Characterization of a new octoploid strawberry breeding population

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Abstract

Among the *Fragaria* species, the cultivated strawberry *Fragaria* × *ananassa* (2n=8x=56) is the most economically valuable crops. After harvest, they are extremely perishable, have a short shelf life, and are susceptible to mechanical injuries and physiological decay due to loss of tissue integrity, sensitivity to fungal diseases, and have a large surface area, which lacks an outer protective rind. Therefore, maintaining high nutritional values in the berry fruit whilst maintaining high fruit quality requires an understanding of the genetic and environmental effects on each trait, and how different traits are associated with each other.

Mapping traits on the linkage map using a Quantitative Trait Loci (QTL) approach is the first step to identify the underlying gene(s) and to explore their effects and interactions. It will improve our understanding of the genetic control of measured traits and facilitates molecular marker development. Therefore, it can be used to improve plant-breeding efficiency at the molecular level, which significantly reduces the breeding time and cost of phenotyping.

The overall aim of this study was to characterise the variation in quality traits among the F1 mapping progeny derived from a cross of Redgauntlet x Hapil (RGxH) strawberry cultivars. These traits include total soluble solids (TSS), titratable acidity (TA), fresh weight, surface colour, firmness, and phenolic

content. This thesis presents two areas of work. First, using a novel high-density single nucleotide polymorphism (SNP) linkage map, phenotyping plant characteristics of RG x H progeny enabled the detection of QTL linked to traits associated with shelf life (at 4 °C). Subsequent QTL analysis highlighted 47 QTL linked to quality traits associated with three post-harvest days in two sequential seasons (2013 and 2014). Among them, three major QTL for fruit lightness (L* value) and TSS/TA ratios were detected in 2013, whereas 17 major QTL were detected in 2014, of which three accounted for >30 % of phenotypic variance. Study results provided additional data on the genetic architecture of fruit quality traits across shelf life at points relevant for strawberry breeding. However, it is still necessary to confirm the stability of the identified QTL resulting from the study findings.

Second, the study evaluated the flavour profiles of seven genotypes of the RGxH F1 strawberry population and their parental lines in order to assess correlations between sensory and instrumental data. Ten trained sensory panellists rated strawberry puree samples on day 1 and day 5 of storage. Thirty attributes were evaluated, including odour, taste, flavour, mouth sensation and aftertaste. Gas chromatography systems were coupled with the solid-phase micro extraction (SPME) method to determine volatility of organic compounds. The results showed a clear separation between desirable attributes, which correlated with most day 1 samples, and undesirable attributes, which correlated with most day 5

samples. Furthermore, the results confirmed the role that volatile compounds (mainly esters, terpenes and aldehydes) and some physical traits (mainly TSS, TA and their ratios) play in sensory perception.

Thesis structure

The findings of this thesis were divided into three results chapters. Two additional chapters, a literature review and description of general materials and methods, precede these three chapters. A general discussion follows the results chapters, and the thesis concludes with a summary discussion of key results. A brief description of each of the thesis chapters follows.

Chapter 1: "Factors affecting the qualitative and sensorial traits of cultivated strawberries (*Fragaria x ananassa*), and how to enhance them"

This chapter introduces the thesis in the form of a literature review, which provides further detail on the study aims and outlines the study objectives and how they were achieved. This review is intended to be published in the journal *Food Science and Technology.*

Chapter 2: "General materials and methods"

This chapter describes the general materials and methods used for all experiments reported in the thesis.

Chapter 3: "The impact of genotypes, storage and cultivation sites on post-harvest strawberry quality"

This first results chapter discusses the changes in strawberry post-harvest quality traits of the progeny of the Redgauntlet x Hapil populations (RGxH). Shelf life storage was studied during two successive harvesting periods (seasons 2013 and 2014) at two different sites in the UK (East Malling and Reading). These results are intended to be published in the journal *Frontiers in Plant Science* along with those from Chapter 4.

Chapter 4: "Mapping QTL underlying fruit quality traits in an F1 strawberry population"

The second results chapter presents the results of segregation of the (RGxH) population for quality traits of strawberries. These results were derived by crossing RGxH, a heterozygous cross that segregates for fruit quality, disease resistance and postharvest traits (Sargent et al., 2009). The chapter also discusses the results concerning the correlation between these traits and their associated QTL over various shelf life lengths using single nucleotide polymorphism (SNP) markers. Along with Chapter 3, these results are intended to be published in the journal *Frontiers in Plant Science*.

Chapter 5: "Sensory analysis of nine genotypes of an F1 strawberry (*Fragaria x ananassa*) and comparison with instrumental analysis"

This third results chapter discusses the findings of the flavour profiles of seven genotypes of the RGxH F1 strawberry and their parental lines at two shelf life points (days 1 and 5) of storage at a commercially standard temperature of 4 °C.

It also identifies correlations between sensory, volatile compound and physicochemical data. The findings presented in this chapter are intended to be published in the journal *Food Chemistry*.

Chapter 6: "General discussion"

This chapter concludes the thesis with a summary discussion of key study results, and identifies the limitations of the study and recommendations for further work on the applications of genomics in strawberry production.

"References"

For the sake of brevity and continuity, references throughout all chapters were listed together in Chapter 7.

"Appendix"

This chapter provides supplementary information and material to the primary chapters.

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List of Abbreviations

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of Variance
H^2	Broad-sense heritability coefficient
CO2	Carbon dioxide
cm	Centimetre
cM	Centimorgan
°C	Degree Celsius
EMR	East Malling Research
=	Equals
FAO	Food and Agriculture Organisation of the United Nations
FW	Fresh weight
GAEs	Gallic acid equivalents
GC	Gas Chromatography
g	Gram
>	Greater than
≥	Greater than or equal to
HPLC	High Performance Liquid Chromatography
IM	Interval Mapping
kg	kilogram
LSD	Least significant difference
<	Less than
≤	Less than or equal to
LG	Linkage Group
LC	Liquid Chromatography
L	Litre
LOD	Logarithm of Odds
MAS	Marker-Assisted Selection
MeOH	Methanol
µl	Microliter
µmol	Micromoles
mg	Milligram

mmol	Millimoles
MW	Molecular weight
MQM	Multiple QTL Mapping
N	Newton unit (force)
r	Pair-wise correlation coefficients
%	Per cent
ph-th	Phenolphthalein
PCR	Polymerase Chain Reaction
PCA	Principal component analysis
QTL	Quantitative trait loci
RAPD	Random amplified polymorphism DNA
rMQM	Restricted Multiple QTL Mapping
RFLP	Restriction fragment length polymorphism
SSR	Simple sequence repeat
SNP	Single nucleotide polymorphism
NaCl	Sodium chloride
NaOH	Sodium hydroxide
SPME	Solid-phase micro extraction
SD	Standard Deviation
SEM	Standard Error of Means
^{o}Brix	Sugar content of an aqueous solution
TA	Titrable acidity
TSS	Total soluble solids

Dedication

To my parents, my wife, my daughter, my brothers and sisters.

Acknowledgment

First of all, I would like to express my great gratefulness and sincere thanks to Allah (God), for providing me the blessings, power and passion to complete this work and achieve my dream.

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Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Rashed Abdulrahman Alarfaj

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Chapter 1 : Factors affecting the qualitative and sensorial traits of cultivated strawberries (*Fragaria x ananassa*), and how to enhance them.

Abstract

Strawberry belongs to the *Rosaceae* family, which contain morphologically diverse flowering plants consisting of more than 3,000 species from approximately 100 genera (Dirlewanger et al., 2002). Economically, *Rosaceae* is the third most important plant family in temperate regions after the *Poaceae* (grass family) and *Fabaceae* (legume family) (Dirlewanger et al., 2002). Strawberries are one of the most highly valued fruits due to their abundance of vitamins, minerals, and phenolic content (Ayala-Zavala et al., 2004; Halbwirth et al., 2006) that give rise to appearance, nutritional and organoleptic qualities that appeal to human consumers. Therefore, there is scope for the continued expansion of the production, which is fundamentally based on fruit quality. Physical (fresh weight, firmness, and colour), chemical (total soluble solids (TSS), titratable acidity (TA), phenolic content) and sensorial (flavour and aroma) traits of strawberry fruits offer means of applying quantitative measurements to represent fruit quality characteristics. These traits are influenced by a number of factors such as genotypic differences, pre- and post-harvest factors, which will eventually affect the value of the fruit. Therefore, strawberry producers are encouraged to develop technical methods to guarantee the sustainable production of strawberry with high quality. To realize these goals, in this review, we independently highlight these qualitative and sensorial traits, how they could be influenced, and how molecular marker applications will help the development of novel breeding approaches.

Key words: Strawberry, flavonoids, Firmness, Colour measurement, TSS, TA, Volatile compounds, Quality and nutritional traits (QTL), Cultivar diversity.

Abbreviations: FW, Fresh Weight; **TSS**, Total Soluble Solids; **TA**, Titratable Acidity; **QTL**, Quantitative Trait Loci.

1.1 Introduction

Cultivated strawberry (*Fragaria* × *ananassa*), which belongs to the family *Rosaceae* in the genus of *Fragaria* (Maas, 1998), is one of the most widely cultivated species in the world in recent centuries (Hancock, 1999). It is the natural hybrid of *F. chiloensis* and *F. virginiana* which is thought to be 300 years old (Darrow, 1966). Among colourful fruits, strawberries are one of the most attractive fruits due to their exceptional flavour and their richness of vitamins, minerals, anthocyanin, flavonoids, and phenolic acids (Ayala-Zavala et al., 2004; Halbwirth et al., 2006). Worldwide, the annual production of strawberry has increased dramatically during the last two decades with a world production in 2014 exceeding 8.1 million tonnes (FAOStat; http:// faostat.fao.org/). Spain is Europe's biggest producer of strawberry after China (1.5 million tonnes) and United state (0.98 million tonnes) producing 0.29 million tonnes every year, Figure 1.1 (FAOSTAT 1993-2013, faostat.fao.org).

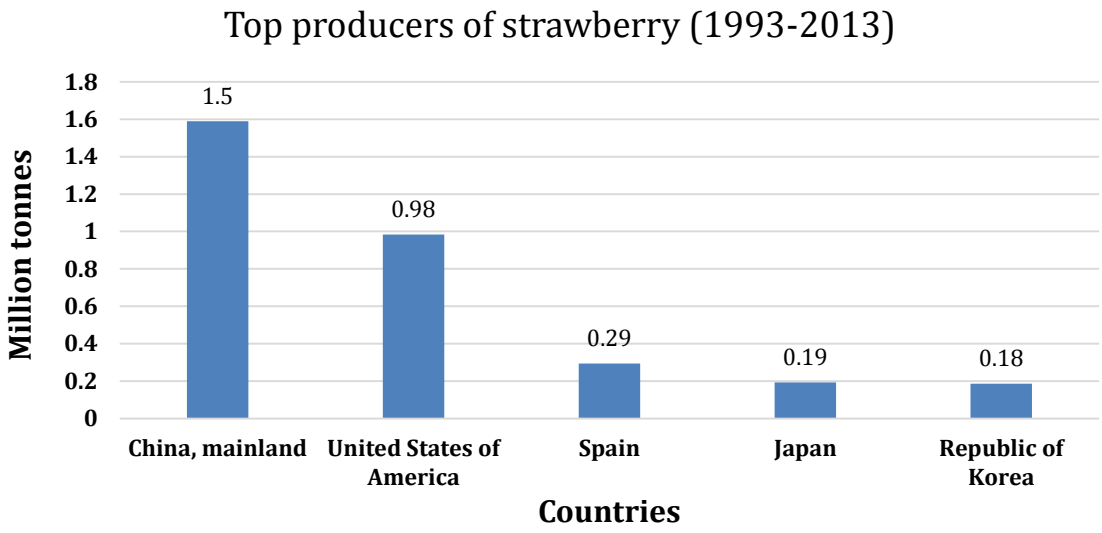


Figure 1.1. World major strawberry producers; Source: (FAO, 2012).

Epidemiological studies indicate that the consumption of polyphenol-rich food, such as strawberry (Halvorsen et al., 2006), linked with the ability to protect human health against many diseases including some cancers, heart diseases (Cook and Samman, 1996; Knee, 2002), neurodegenerative diseases (Spencer, 2009), attenuate cognitive decline and neuronal dysfunction (Vauzour et al., 2008). This might be due to their antioxidant capacity activity against cellular oxidation reactions (Capocasa et al., 2008; Rice-Evans et al., 1996; Wang and Lin, 2000), although most researchers now believe that these compounds act positively through a mechanism that is not only associated with the antioxidant properties (Giampieri et al., 2015; Schroeter et al., 2006) .

From the botanical point of view, strawberry is classified as an herbaceous perennial plant that survives for several years and can reproduce both sexually and asexually. It consists of a stem/crown found in the soil level from which arises

leaves, roots, runners/stolons and inflorescences (Hancock, 1999; Maas, 1998; Taylor, 2002) (Figure 1.2). In commercial practice, runners are vegetatively propagated by pinning down the daughter plant which produce its own root system and develop into an independent plant. This practice typically allows the propagation of genetically identical (clonal) plants with all the favourable characteristics of the mother plant.

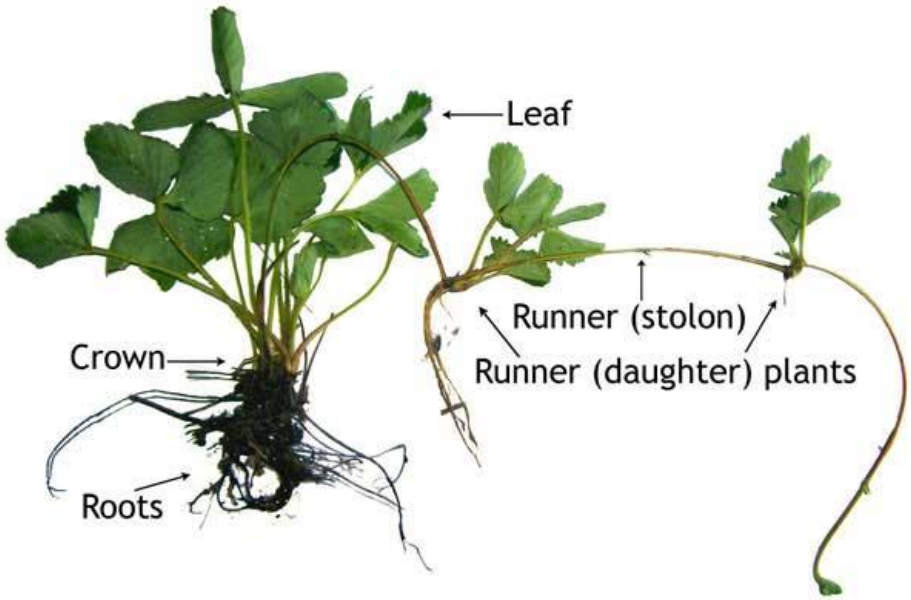


Figure 1.2. The structure of a strawberry plant (Reproduced from Roper 1991).

Axillary buds are developed at the base of the leaves and produce runners and branch crowns which are fundamentally shoots. The fleshy strawberry fruit (seeded fruit) is an enlarged receptacle with huge number of achenes (popularly called seeds) at the surface, each strawberry is produced from a single white

flower bearing many stamens. The achenes emerge from the base of each pistil and link the seed and ovary tissues (Perkins-Veazie, 1995).

Based on its photoperiods and environmental control of flowering, strawberry classified to three types: long day plants "Ever-bearing", those produce few runners and initiate flower buds under long days, short day plants "June-bearing", those initiate flower buds under short days, and neutral day plants (Hamano et al., 2008; Hancock, 1999; Nishiyama and Kanahama, 2009).

Strawberry characteristics are known to be influenced by the environmental factors such as temperature, light, moisture, and soil. Therefore, it is important to develop cultivars that fit with the environmental conditions of desired specific area (Martínez-Ferri et al., 2014). For this reason, the aim of the worldwide breeding programs is to develop strawberry cultivars well-adapted to the specific environmental conditions where they are going to be cultivated.

After harvest, strawberries are extremely fragile and perishable, have a short market shelf life, and are susceptible to mechanical injuries and physiological decay due to their firmness loss, their sensitivity to fungal diseases, and large surface area which lacks the outer protective rind (Bitencourt De Souza et al., 1999). Post-harvest quality traits or otherwise known as consumer quality traits, such as colour, firmness, flavour, and phenolic content, are becoming very important traits for breeders and consumers (Lerceteau-Kohler et al., 2012; Sargent et al., 2009, 2012, Zorrilla-Fontanesi et al., 2012, 2011). These traits are

influenced by different factors such as genotype, pre-harvest and post-harvest environments (Crespo et al., 2010; El Hadi et al., 2013; Forney et al., 2000; Hakala et al., 2002; Soria et al., 2008; Wang and Lewers, 2007).

Strawberry quality and nutritional traits associated with long storage and high nutritional value are major topics of several current breeding programmes (Capocasa et al., 2008). Thus, there is a need in breeding programmes to enhance these traits in the fruit to increase consumption as part of a healthy diet and make the fruit appeal to the widest possible range of consumers. However, the challenge for the breeders is to maintain high nutritional values in the berry fruit whilst maintaining an outstanding fruit quality. Therefore, they require knowledge of the genetic and environmental regulation of each single trait, what affects variation between genotypes and how different traits are associated. This target could be achieved by the combination of molecular marker and trait data to help locating the gene responsible and to explore their effects and interactions. Mapping traits on the linkage map using a Quantitative Trait Loci (QTL) approach is the first step to identify the underlying gene(s) and to explore their effects and interactions. These may be gene(s) which directly regulate the trait e.g. genes involved in synthesis of phytochemicals, or gene(s) which act indirectly to regulate turnover, induction of transcription factors, or response to the environment. Thus, a QTL approach is more powerful than just looking at gene(s) thought to be involved in biosynthesis as it enables a number of different control points for each trait of

interest to be identified. In this review, the aim was to investigate in detail these quality traits and how they can be improved.

1.2 **Polyphenols**

Phenolic compounds are substances which possess an aromatic ring bearing one or more hydroxyl groups (Harborne, 1984; Ho, 1992; Macheix et al., 1990) and are distributed widely in the plant kingdom with more than 8000 phenolic structures currently known (Kosar et al., 2004). They exist in almost all plant parts including leaves, roots, woods, flowers, seeds (Markham, 1982). These compounds are classified into different groups including simple phenols and phenolic acids, hydroxycinnamic acid derivatives, and flavonoids (Cartea et al., 2011; Ho, 1992; Kosar et al., 2004). The free forms of these compounds are very rarely exist in plants, however they are usually either esterified, etherified, or glycosylated (Daayf and Lattanzio, 2008; Macheix et al., 1990; Markham, 1982). The glycosylated form, which is the abundantly occurring form, develops from *glucosyltransferase* activity and help to these compounds to be less reactive and more soluble (Markham, 1982).

1.2.1 Function and use of polyphenols

In plants, phenolic compounds may cause undesirable consequences through the action of *polyphenoloxidase* (PPO) that catalyses the enzymatic browning reaction of phenolic acid resulting in unwanted colour, flavour and loss of nutrients in fruits and vegetables (Jia et al., 2016). However, phenolic compounds

are also important for a multiplicity of beneficial functions in plants and humans. They have long been recognised as playing multiple roles in plants including attracting insects for seed dispersion and pollination (Carbone et al., 2009), pigmentation (Harborne, 1984), growth, defence against pathogens and insects, UV protection, and many other functions (Asami et al., 2003; Davies and Schwinn, 2003; Gould and Lister, 2006). In humans, a growing body of information suggests that regular consumption of food rich in phytochemicals have a multiplicity of beneficial effects on human health including reducing the risk of chronic disease; such as cardiovascular and cancer diseases (Daayf and Lattanzio, 2008; Hannum, 2004), potential to promote memory, learning and cognitive functions (Spencer, 2009; Vauzour et al., 2008).

The health benefit of phenolic compounds in human protection attributed to their biological properties. Giampieri et al. (2012) stated, "The hypothesized health benefits related to strawberry consumption include their role in the prevention of inflammation, oxidative stress and cardiovascular disease (CVD), certain types of cancers, type 2 diabetes, obesity, and neurodegenration". The biological and functional activities of phenolic compounds have been alos attributed to other pathways involved in cellular metabolism and survival (Forbes-Hernandez et al., 2015; Giampieri et al., 2014). Their role in reducing cardiovascular risk has been attributed to their ability to increase the bioavailability of nitric oxide and lowering blood pressure (Schroeter et al., 2006; Spencer, 2009). They could also

attribute as anti-carcinogens as they may play an important role to reduce oxidative damage to DAN and reduce the bioavailability of carcinogens (Stavric et al., 1992; Taie et al., 2008; Wang and Lewers, 2007). On the other hand, the impact of flavonoids on the brain was recently attributed to their ability to exert neuroprotecive actions through their interactions with critical neuronal intracellular signalling pathways pivotal in controlling neuronal survival and differentiation, long-term potentiation (LTP) and memory (Spencer, 2009, 2007; Williams et al., 2004).

1.2.2 Strawberry polyphenols

1.2.2.1 Phenolic acids

Ellagic acid is the major phenolic acid in strawberry forming almost 51 % of phenolics, followed by p-Coumaric acid, Hydroxy-benzoic acid, and then quercetin (Häkkinen et al., 1999, 1998; Häkkinen and Törrönen, 2000; Kosar et al., 2004). Ellagic acid consists of a complex planar unit having four hydroxyl groups and two lactones groups (Barch et al. 1996), Figure 1.3. Its content in strawberry and raspberry is approximately three times higher than its content in other fruits or nuts (Kosar et al., 2004; Williner et al., 2003), making strawberry a good target for further improvement. Levels of ellagic acid found in the literature vary greatly depending on many factors including genotypes, cultivation conditions, ripeness and temperature (0.22 to 46.5 mg/100 g FW) (Häkkinen et

al., 1999, 2000; Häkkinen and Törrönen, 2000; Kosar et al., 2004; Wang, 2007; Williner et al., 2003).

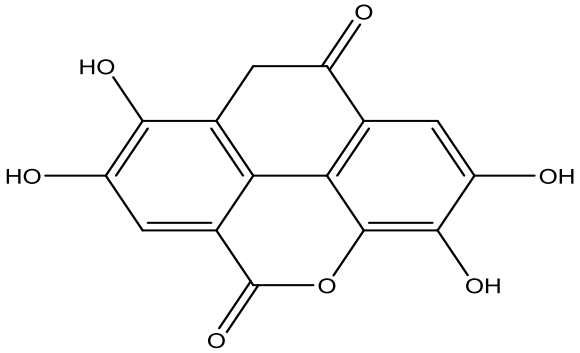


Figure 1.3. The chemical structure of ellagic acid (Adapted from Barch et al., 1996).

1.2.2.2 Flavonoids

The most numerous group of phenolic compounds in food are flavonoids (Ho, 1992) which are a group of secondary metabolites that are distributed widely in plants (Macheix et al., 1990) and derived from phenylalanine and tyrosine (Pereira et al., 2009). They consist of an aromatic ring bearing one or more hydroxyl substituents, for example functional derivatives including esters, methyl ethers, and glycosides etc, shown in Figure 1.4. They are generally divided into different groups including flavonols, flavones, flavanones, flavanoles, isoflavonones, anthocyanins (Ho, 1992).

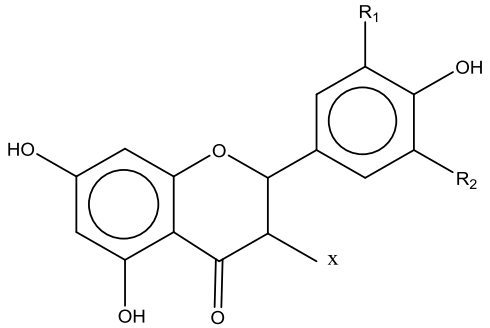
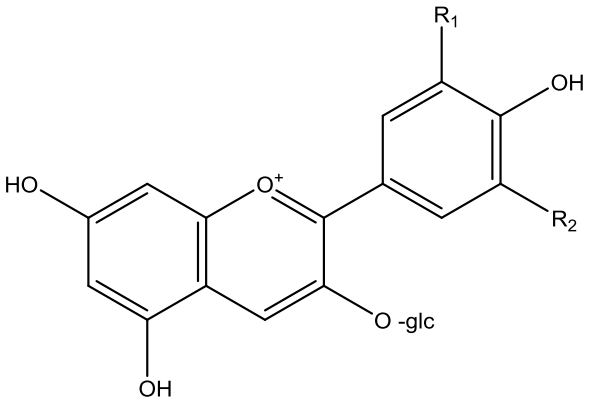


Figure 1.4. The chemical structure of the basic flavonoid (Adapted from Hertog et al., 1992).

1.2.2.2.1 *Anthocyanins*

Anthocyanins are a major group of flavonoids synthesized naturally from a non-polar amino acid called phenylalanine through several enzymatic reactions (Carbone et al., 2009). They are the glycosides of anthocyanidins those known as the main food colorants in the plant kingdom (Ho, 1992) those can be identified by HPLC at 520 nm wavelength (Seeram et al., 2006). In plants, anthocyanins are frequently found to be linked to sugars at the C3 hydroxyl group and forming a glycosidic bond which provides stability and water solubility (de Pascual-Teresa et al., 2010; Gao and Mazza, 1995). In strawberry, pelargonidin, cyanidin, and their derivatives are the main pigments with 90 % formed of *pelargonidin-3-glucosidase* (Hancock, 1999; Kosar et al., 2004), Figure 1.5. It is well-known that sugars are the initial precursor of the anthocyanin biosynthesis (Hrazdina et al., 1984; Ruhnan and Forkmann, 1988; Teusch et al., 1987).



R1	R2	Anthocyanidin
H	H	Pelargonidin
OH	H	Cyaniding

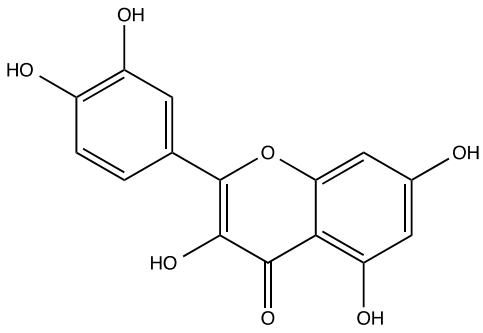
Figure 1.5. The chemical structure of anthocyanins.

Based on the study conducted by (Beking and Vieira, 2011) to estimate flavonoid consumption, the daily flavonoid intake (anthocyanidins, flavonols, flavanols, flavanones, and flavones) in the UK and Ireland was 182 and 177 mg/day, respectively. While in the US, the daily intake of total anthocyanins has been estimated to be as little as 12.5 mg/day depending on the diet (Wu et al., 2006), much less than estimates published in the 1970s that put average daily anthocyanin intake at 180-215 mg/day (Kühnau, 1976). Among the European countries, their daily intake of anthocyanidins ranged between 19.8 to 64.9 mg/day for men, whereas for women between 18.4 – 44.1 mg/day (Zamora-Ros et al., 2011). However, the daily intake of anthocyanin may vary broadly among different populations, different regions and seasons, and among individuals with different education, financial status, and culture (Wu et al., 2006). Interest in anthocyanins has increased immensely during the last decade because of their important role in health promotion and disease prevention. However, little is

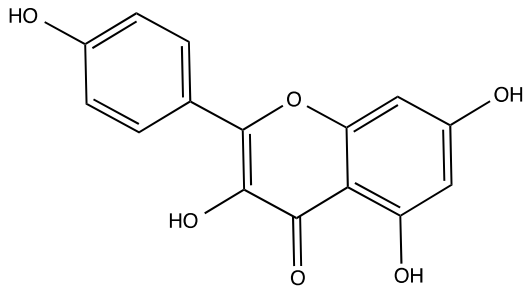
known about their absorption process in the gut or in which tissues they might exist (Lila, 2004). To our knowledge, there have been no population-based studies that proposed the flavonoid amounts required daily to elicit a health benefit.

1.2.2.2.2 *Flavonols and Flavanols*

According to Häkkinen et al (1999), the main flavonol among the 19 berries, especially in strawberry, was quercetin, followed by kaempferol which presented in quite low amounts, whereas the main flavanol in strawberry is catechin (Figure 1.6). The daily combined intake of flavones, flavanones, and flavonols in the UK and Ireland was 60 and 69 mg/day, respectively (Beking and Vieira, 2011). Previous study indicated that the levels of quercetin range from 0.3 to 5.3 mg/100 g FW, and the levels of kaempferol are ranging from very small amounts to 0.9 mg/100 g FW (Wang, 2007).



Quercetin



Kaempferol

Figure 1.6. The chemical structure of Quercetin and Kaempferol (Adapted from Ciolino et al., 1999).

1.2.3 Factors affecting phenolic content in strawberry

Two key factors have the potential effect on polyphenol presence and content: genetics and environment. However, within this different factors could also affect the phenolic content and antioxidant capacity of berries including maturity of fruits at harvest stage (Kalt et al., 1999), pre-harvest environmental conditions, post-harvest behaviour and storage conditions (Häkkinen and Törrönen, 2000; Wang, 2007).

1.2.3.1 Genetic factors

The effect of cultivars on flavonoid content among different fruits and vegetables including onions, pear, red raspberry, apricot, grape (Summarised in Häkkinen and Törrönen, 2000), and strawberry were reported (Aaby et al., 2012; Camargo et al., 2011; Cardeñosa et al., 2016; Josuttis et al., 2012). The genetic variation of different cultivars is one of the main factors affecting the phenolic content in strawberry (Atkinson et al., 2006; Cardeñosa et al., 2016; Häkkinen and Törrönen, 2000; Meyers et al., 2003; Wang, 2007). This could suggest that the phenolic content of strawberry fruits is mainly under the genetic control and understanding the regulation mechanism of its synthesis will be helpful and provide meaningful ideas in future for strawberry breeders.

1.2.3.2 Pre-harvest factors

The content of phenolic compounds in strawberry varies as results of different factors, some of which are pre-harvest conditions that encompass pre-harvest

temperature (Wang and Zheng, 2001), cultivation system (Camargo et al., 2011; Cocco et al., 2015; Jin et al., 2011), cultivation site (Häkkinen and Törrönen, 2000), and UV transparency of protected growing systems (Tsormpatsidis et al., 2007). Previously, the impact of temperature was reported by Wang and Zheng who found that the content of phenolic acid, flavonols, and anthocyanins are constantly increased with increasing temperature (Wang and Zheng, 2001). This rise results from increasing the activity of *phenylalanine ammonia-lyase* (PAL) and *chalcone synthase* (CHS), the main two enzymes in the phenylpropanoid pathway (Ariza et al., 2015; Josuttis et al., 2012; Wang and Zheng, 2001).

Cultivation systems may also influence the content of phenolic compounds in strawberry. For example, organically grown strawberry have a higher total phenolic compounds compared with conventionally grown strawberry (Camargo et al., 2011; Cocco et al., 2015; Häkkinen and Törrönen, 2000; Jin et al., 2011). This is probably resulted from the stress (biotic and abiotic) of strawberry plant that takes place where herbicides, pesticides and insecticides are not applied which could induce the synthesis of phenolic compounds (Häkkinen and Törrönen, 2000; Zhang et al., 2007). Cardeñosa et al. (2016) has also shown that total phenolic composition (mainly flavonols) increased when berry fruits where grown under open-field conditions comparing with those grown under plastic tunnel. On top of all this, cultivation site also have a great impact on the amount of phenolic compounds as reported in previous studies (Cocco et al., 2015;

Häkkinen and Törrönen, 2000; Krüger et al., 2012). Ultimately, there was a significant difference in phenolic content between cultivars planted at different sites, however this also depended on other factors including genotypic variation.

Likewise, the effect of fruit maturity/ripening stage and light intensities were also determined (Wang et al., 2009). During fruit ripening, anthocyanin content was found to increase (Aaby et al., 2012) while total phenolic content decrease (Wang et al., 2009). Light intensity has been reported to influence the accumulation of phenolic compounds in strawberry (Wang et al., 2009). Strawberry grown with high intensity light (exposed to photosynthetically active radiation level of 56 ± 0.2 μmol m^{-2} s^{-1}) had higher anthocyanin and total phenolics than others grown in low intensity (31 ± 0.2 μmol m^{-2} s^{-1}), which however depends on maturity stage as well (Wang et al., 2009).

1.2.3.3 Post-harvest factors

As pre-harvest conditions influence the quality of strawberry including phenolic content, post-harvest conditions could also contribute to the variation of the phenolic content.

1.2.3.3.1 *Storage conditions*

One of the main post-harvest factors affecting phenolic content of fruits is storage condition. Number of post-harvest conditions during storage including controlled atmosphere (CA), low temperature, and high CO_2 concentration have the ability to maintain post-harvest quality of fruits and extend the shelf-life (Gil et al., 1997;

Pelayo et al., 2003), but some conditions such as low temperature (< 0 °C) and high CO_2 (+20 kPa CO_2) with low O_2 concentrations, would lead to lower content of anthocyanin (Cordenunsi et al., 2005; Holcroft and Kader, 1999). Anthocyanin content of internal tissues of strawberry was also reported to decrease while stored at controlled atmosphere (CA) which could suggest the inhibition of PAL (Holcroft and Kader, 1999). These results could suggest that strawberry stored at controlled atmosphere (CA), such as low temperature with high CO_2 concentration, would have better quality to those stored at high temperatures in air, but very low temperature with high CO_2 concentration have an adverse effect on anthocyanin content.

1.2.3.3.2 *Temperature*

Temperature is probably the most important factor affecting post-harvest quality of fruit, and this might be because of its considerable consequences on rates of biological reactions and microbial growth (Ayala-Zavala et al., 2004). In general, the decrease of temperature slows metabolism and development. A study conducted to evaluate the content of phenolic compounds in strawberry kept at 0 °C, 5 °C, and 10 °C concluded that fruit stored at 5 °C, and 10 °C had higher total phenolics and anthocyanin than those at 0 °C (Ayala-Zavala et al., 2004). Cordenunsi et al., (2005) found that anthocyanin showed an increase during storage, but this increase was significantly influenced by temperature as the rate increased with increasing temperature. He also found that storage at different

temperature conditions had no effect on flavonols, ellagic acid, and total phenolics (Cordenunsi et al., 2005). These results could suggest that the synthesis of flavonols or ellagic acid cannot take place after harvesting. Nevertheless, strawberry stored at low/cold temperature (0 °C) would have better quality to those stored at high temperatures (5 °C and 10 °C) but low content of total phenolics and anthocyanin.

1.3 **Post-harvest quality of strawberry**

The term "quality" can be described in many different ways, for example; "fitness for use" or "quality to meet the expectations of customers" (Knee, 2002; Rasing et al., 2003). Quality traits of fresh fruits such as firmness, colour, size, shape, flavour and aroma represent the common characteristics for consumers which might play an important role to dissuade them from consuming fruits if these traits are poor (Bénard et al., 2009; Gunness et al., 2009). These traits could be measured either by instruments or sensory measurements.

1.3.1 Physicochemical traits

1.3.1.1 Firmness

According to ISO Standard 5492 (1992), texture described as "the perceptible mechanical traits of food in mouth by different receptors including mechanical, tactical, visual and auditory receptors" (Cited by Costell and Duran, 2009). It is known as one of the most perceptible trait that might be available to consumer for assessing strawberry quality. In general, textural analysis of fruits and vegetables

is one of the main indicators of fruit quality and could provide a wide range of information that would help to understand the mechanical properties of the fruit and their resistance to injuries (Cited by Gunness et al., 2009). This trait could be influenced by several factors, some of which are temperature, cultivation system (Soria et al., 2008), size of strawberry, season of harvesting, and ripeness stage (Rasing et al., 2003).

The primary cell well of plants are mainly consist of polysaccharides, low amount of glycoproteins, and phenolic esters (Figueroa et al., 2010; Koh and Melton, 2002; Vicente et al., 2005). Pectin, cellulose, and hemicellulose are the main cell wall polysaccharides linked to fruit softening during ripening, however the biochemical basis of strawberry cell wall degradation is still not been fully understood (Molina-Hidalgo et al., 2013; Rees et al., 2012). The general consensus is that the degradation of the middle lamella of the cell wall is the effective reason behind the softening of fruits during ripening (Rees et al., 2012). This degradation is mainly controlled by specialised enzymes including *Endo-1,4-β-d-glucanase* (EGase), *β-Xylosidase* (B- Xyl), *polygalacturonase* (PG), and *pectin methylesterase* (PME) (Figueroa et al., 2010; Koh and Melton, 2002; Molina-Hidalgo et al., 2013; Vicente et al., 2005). In strawberry, this is due to the solubilisation of cell wall pectin (polyuronides), forming up to 60 % of cell wall polysaccharides mass, rather than hemicellulose and cellulose solubilisation, which are chiefly responsible to give solidity to the cell wall (Ali et al., 2011;

Figueroa et al., 2010; Molina-Hidalgo et al., 2013; Vicente et al., 2005). However, this degradation could be controlled by applying some treatments such as refrigeration or/and heat treatments which inhibit the activity of these enzymes and then delay the softening (Vicente and Costa, 2006).

1.3.1.2 Colour

Colour of fresh fruit is an important factor in consumer satisfaction and could influence repeat consumption of the food. It is used as an indicator of maturity in many fruits, including strawberry. Different factors have the ability to influence the colour of strawberry fruit. Some of which are storage conditions (Ayala-Zavala et al., 2004; Miszczak et al., 1995), ripening progress (Gil et al., 1997), genotype, and harvesting and handling processes (Wang and Zheng, 2001).

1.3.2 Factors influencing physicochemical traits

Different factors were reported to influence fruit quality including genotype, temperature, light intensity (Summarised by Hancock, 1999), cultivation system, day length (Soria et al., 2008), and crop protection chemicals (Camargo et al., 2011). Post-harvest quality could be also influenced by other factors, including maturity at harvest, humidity, level of absorbance and metabolism of mineral nutrients by plants, storage conditions, poor pollination and occurrence of damage to the achenes caused by insect and diseases (Häkkinen and Törrönen, 2000; Hancock, 1999; Kader, 1997; Knee, 2002; Kosar et al., 2004). Taken together, such factors seem to have a direct impact on the physiochemical traits of

strawberry, signalling the need to adapt the appropriate practice in the conventional breeding programmes.

1.3.2.1 Pre-harvest factors

A major decrease in firmness (Civello and Martínez, 1997; Nunes et al., 2006) and colour change of strawberry (Civello and Martínez, 1997; Miszczak et al., 1995) were reported during ripening. Such change in colour could attributed to the accumulation of anthocyanins and decrease of chlorophyll synthesis during ripening process (Cited by Civello and Martínez, 1997). Strawberry colour was reported to become darker and more red when the temperature became warmer (Miszczak et al., 1995; Wang and Zheng, 2001). Fruit colour was also reported to be influenced by light intensity as the colour development was greater in fruits harvested at red, pink, and white stages of development and stored in light comparing to fruits stored in dark (Miszczak et al., 1995).

Additionally, Soria et al. (2008) reported that strawberry grown under small plastic tunnels were firmer than those grown under the long ones. They found that the temperature was higher under the large tunnels compared to the small tunnels in both seasons which could suggest that high temperature led to high fruit softness and damaged the tissue.

An essential nutrient is a nutrient required in a certain amount to maximize plant performance (Agulheiro-Santos, 2008). Nitrogen and calcium are the most important nutrients that might affect plant growth and post-harvest quality of fruit.

It is well-known that the increase of calcium content of fruit and nitrogen content of soil could also increase the post-harvest-life (Knee, 2002). Calcium can play a major role in delaying the softening of fruits, as it is an important part of the cell wall structure, by slowing the degradation of cell wall polymers (Cheour et al., 1991; Fallahi et al., 1997). This could occur by the formation of cationic bridges between pectic acids or between pectic acids and other polysaccharides through the binding of calcium to pectins, and hence reduce the susceptibility of the cell wall to the action of pectolytic enzymes (Conway et al., 1994; Knee, 2002).

Nitrogen is also essential for obtain a good quality as it plays an important role in a cell's biochemical machinery. The use of nitrogen usually allow plants to grow, develop and produce maximum yields as well as obtain a high quality fruits with required characteristics including colour, flavour, firmness, and nutritional composition (Ritenour, 1999; Sun et al., 2012). Strawberries have been analysed to evaluate the effect of nitrogen levels on postharvest quality. Four levels of nitrogen were examined: type 1 was without nitrogen, type 2 was 5 g/cm^2, type 3 was 10 g/cm^2, and type 4 was 15 g/cm^2. An obvious difference was noticed between type 1 (without nitrogen) and type 2 (5 g/cm^2) which gave a considerable increase of quality, whereas, no significant differences were noticed between type 3 (10 g/cm^2) and type 4 (15 g/cm^2) (Agulheiro-Santos, 2009). Benard et al. (2009) found that lowering supply of nitrogen from 12 to 6 or 4 mM NO_3^- could affect secondary metabolites, decrease vegetative development, and increase sugar

content in tomato. Similarly, calcium deficiency may lead to many disorders associated with fruit quality; for example, bitter pit in apples, cork spot in apples and pears, and red blotch in lemons (Knee, 2002). However, little information is available regarding the effects of nitrogen and calcium levels on flavonoid content and shelf life quality of strawberry.

1.3.2.2 Post-harvest factors

The effect of storage temperature on strawberry post-harvest life has been studied by Ayala-Zavala et al. (2004). They had three different storage conditions (0 °C, 5 °C, and 10 °C) and showed that 0 °C was the best temperature to maintain the excellent overall quality of this fruits (Ayala-Zavala et al., 2004). They found that high temperature storage led to high fruit softness and damaged the tissue, decreased the content of TSS and reduced the shelf life by increasing the rate of fruit development, which in turn reducing their quality and attractiveness (Cited by Hancock, 1999). Thus, low temperature storage of strawberry fruits could help to preserved fruit firmness which decrease the susceptibility to decay and then improve the shelf life by slowing the respiratory metabolism (Hansawasdi et al., 2006).

Many techniques can be used to maintain and extend the post-harvest quality of strawberry, some of which are refrigeration, hot air treatment (Vicente et al., 2005), modified and controlled atmosphere (Pelayo et al., 2003). It has been found that heat treatment for a specific time (e.g. 45°C for 3 h) can delay fruit softening

by the inhibition of specific enzyme such as *Endo-1,4-β-d-glucanase* (EGase) and *β-Xylosidase* (B- Xyl) which delays hemicellulose and pectin degradation (Figueroa et al., 2010; Vicente et al., 2005). In general, the combined effect of heat treatment on these enzymes could reduce the solubilisation of pectin and then delay the softening (Vicente et al., 2005). Similarly, modified atmosphere (CO_2-enriched) could also improve the yield (Sun et al., 2012) as well as some important quality traits of strawberry including TA, TSS, firmness, colour, and reduce decay incidence (Pelayo et al., 2003; Zhang and Watkins, 2005). A correlation between elevated CO_2 and increased total sugar levels was observed by Sun et al., (2012). Although, the mechanism of effects of CO_2 on strawberry quality is yet unknown, strawberry firmness enhancement could be due to the changes of apoplastic pH (Harker et al., 2000). This would in turn allow promoting the precipitation of soluble pectins and then enhance cell-to-cell bonding. However, not all quality traits can be preserved to the same extent.

1.4 **Sensorial trait**

Flavour is described as sensory impression originated as a result of a material taken in the mouth (sweetness, sourness, bitterness, and saltiness) and determined mainly by the senses of taste and smell (Knee, 2002; Zabetakis and Holden, 1997). Flavour perception can be mainly identified by the role of the human olfactory system which has the ability to identify and distinguish volatile compounds of

different molecules (Sankaran et al., 2012). Sankaran and his colleagues highlighted three key components of the olfactory system. These are:

1) Olfactory region (nose)

2) Olfactory receptors

3) Regions of olfactory signalling

Aroma can reach the olfactory system through two pathways, those are orthonasal (sniff) and/or retronasal (taste) (Ruijschop et al., 2009; Smelling, 2004). Human olfactory mechanisms are quite complex, the primary odorant information is processed in the olfactory receptors and then sent to the olfactory bulb which distributes the information to other parts of brain in order to identify and detect the flavour (Firestein, 2001; Sankaran et al., 2012).

Many different studies on flavour assessment of strawberry have been conducted during last couple of decades (El Hadi et al., 2013; Schwieterman et al., 2014; Song and Forney, 2008; Tressl et al., 1975; Yamashita et al., 1977; Zabetakis and Holden, 1997). According to Azodanlou et al., (2003), almost 30 % of strawberry consumers are often disappointed with the quality, including flavour. Volatile (aroma compounds) and non-volatile (sugar and organic acid) compounds are believed to be responsible for strawberry flavour.

1.4.1 Volatile compounds

Volatile compounds are formed among high number of fruits as an indicator of fruit ripening and they are responsible for the unique flavours of fresh fruits. These compounds are classified in five classes of chemicals as major flavour contributors in fruit: ester, alcohol, aldehydes, ketones and terpenoids (Kader, 1997), those have already been identified in both cultivated and wild strawberry (Zabetakis and Holden, 1997). These compounds are often present in small quantities (trace amounts), but have a major effect on fruit quality (Kader, 1997). They are produced as a result of an enzymatic reaction such as the esterification of 1-pentanol or non-enzymatic reactions such as the reaction of an alcohol with an acid (Yamashita et al., 1977). They have long been recognised as playing multiple roles in plants including attracting insects for seed dispersion and pollination (Rowan, 2011), revealing that fruit are ripe and ready for seed dispersal and modulating systemic acquired resistance to pests and diseases (Cited by Rowan, 2011).

For strawberry aroma, more than 350 volatiles have been identified, making it one of the most complex fruit aroma profiles, but only small portion of them were reported to be important for the strawberry flavour (Azodanlou et al., 2003; Bood and Zabetakis, 2002; El Hadi et al., 2013; Forney et al., 2000; Hakala et al., 2002; Schwieterman et al., 2014). Their relative contribution to aroma depends on their concentrations in strawberry and their odour threshold (Forney et al., 2000;

Zabetakis and Holden, 1997). The odour threshold is defined as "the first concentration at which all panel members can recognize the odour" (Leonardos et al., 1969). From these two values (concentration and threshold) an odour value, which is defined as the ratio of concentration of compound to its threshold value, can be calculated and the greater odour value is the greater contribution to flavour (Forney et al., 2000; Zabetakis and Holden, 1997). Furthermore, volatile compounds are derived from metabolism processes of different compounds including lipids, amino acids, phenolic and terpenoid (Knee, 2002), and their concentration depend on cultivar and ripening stage of strawberry (Jetti et al., 2007).

Esters and furanones are the main strawberry flavour compounds (Song and Forney, 2008; Zabetakis and Holden, 1997). Recent studies reviewed the volatile compounds responsible for flavour among apples (Fellman et al., 2000), strawberries (Azodanlou et al., 2003; Forney et al., 2000), melons (Lignou et al., 2014; Song and Forney, 2008), pear, banana, citrus, grape and pineapple (reviwed by El Hadi et al., 2013), and they found that esters were the common group of volatiles present in these fruits.

Many papers focused on the production of ester group as it is the major group of volatiles existing in many fruits generally and strawberry specifically (El Hadi et al., 2013; Pelayo et al., 2003; Perez et al., 1992; Song and Forney, 2008). *Alcohol acyltransferase* (AAT) is the primary enzyme for ester formation which catalyses

the esterification of alcohols and carboxylic acids (Forney et al., 2000; Pérez et al., 2002). This reaction is a very simple reaction which known as a coenzyme-A-dependent reaction (Bood and Zabetakis, 2002);

$$\text{Alcohol} + \text{Acyl-CoA} \xrightarrow{\text{AAT}} \text{Ester}$$

Because of its importance to strawberry flavour, the biosynthesis of ester, illustrated by Zabetakis and Holden (1997) in Figure 1.7, was commonly studied. *Pyruvate decarboxylase* (PDC) and *alcohol dehydrogenase* (ADH) were also reported as an important enzymes in ester formation (Zabetakis and Holden, 1997). PDC is responsible for removing the carbon dioxide from the pyruvate and providing aldehydes, while ADH, an NAD(P)-dependent enzymes, responsible for converting the aldehyde to alcohol which is a major substrate of ester formation (Pérez et al., 2002; Zabetakis and Holden, 1997).

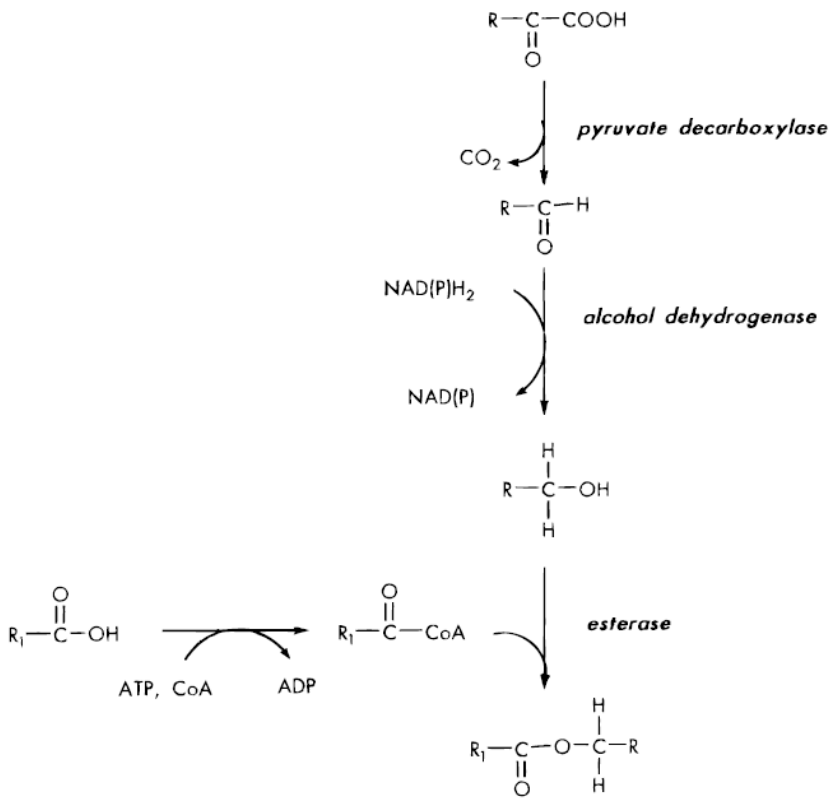


Figure 1.7. The formation of esters in strawberry fruits (Adapted from Zabetakis & Holden 1997).

Esters including methyl and ethyl butanoate, butyl acetate, methyl and ethyl hexanoate, linalool, γ-decalactone and 2,3-butanedione formed approximately 90 % of the volatiles among ripe strawberries as the most abundant class of strawberry volatile compounds (Azodanlou et al., 2003; Jetti et al., 2007). Another class of compounds which may comprise up to 50 % of strawberry volatiles is furanones including 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) and its methyl derivative 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifurane) (Forney et al., 2000; Jetti et al., 2007). Esters were reported to be responsible for the "fruity and floral aroma" (Forney et al., 2000), while furanone compounds were reported to

be responsible for the specific aroma of strawberry such as “sweetness”, “caramel” and “refreshing fruitiness” (Jetti et al., 2007; Perez et al., 1996). Additionally, aldehydes and alcohols including hexanal, trans-2-hexenal and cis-3-hexen-1-ol are also important groups of volatiles for unripe strawberry aroma (Jetti et al., 2007).

1.4.2 Non-volatile compounds

The main components of fruit organoleptic quality are flavour, sweetness, and acidity. The content of sugar, which is positively correlated with sweetness, and acidity, which is typically linked with sourness, are the most important factors influencing strawberry flavour (Knee, 2002; Zabetakis and Holden, 1997). High soluble solids (TSS) and total soluble solids/titratable acidity ratio (TSS/TA ratio) are normally associated with best flavour and fruit quality (Bénard et al., 2009; Mikkelsen, 2005). It was proposed that maximum 0.8 % of titratable acidity (TA) and minimum 7 ºBRIX of TSS are the required values for an acceptable flavour (Pelayo et al., 2003). Thus, fruit organoleptic quality is highly linked, up to a certain extent, with lowering acid content and increasing soluble solid content.

One of the main soluble components in plants is sugar that is important for plant growth and metabolism as an energy source. Sucrose, fructose, and glucose are the main soluble sugars of ripe strawberry (Knee, 2002). During ripening, sucrose is the abundant sugar which is hydrolysed into glucose and fructose (Fait et al., 2008). Likewise, organic acids are important flavour components as they can

affect strawberry flavour positively, by forming the required acids for good flavour, and negatively by forming off-flavours (Cited by Zabetakis and Holden, 1997). TA is considered as a measure of buffering capacity of fruit, which generally expressed as a percent citric acid. The main organic acid in strawberry fruits is citric acid, forming almost 60-70 % of total acid content (Crespo et al., 2010; Kafkas et al., 2007; Watson et al., 2002). Although, the biosynthesis pathways for sugars and volatiles in fruit are not fully understood, sugar compounds are formed as a result of the photosynthesis pathway while acids are formed through series of reactions through the tricarboxylic acid (TCA) cycle (Knee, 2002).

1.4.3 Factors influencing strawberry flavour

Different factors can affect flavour through influencing the composition of specific chemical constituents including volatile compounds, TSS and/or TA in fruit generally and strawberry specially. Some of these factors are cultivar variation, maturity stage, irrigation and fertilization, and post-harvest handling.

1.4.3.1 Genetic factors

Cultivar variation is one of the factors influencing the volatile content (El Hadi et al., 2013; Forney et al., 2000; Hakala et al., 2002; Miszczak et al., 1995; Rees et al., 2012), as well as TSS and TA content of the fruit (Crespo et al., 2010) and thus can affect the taste quality of the product. El Hadi et al., (2013) reviewed the aroma compounds of different fruits and stated that the concentration of major

volatiles in grape, apple, and strawberry varied according to the genotype. In strawberry, differences have been found between the cultivated and wild types (Reviewed by El Hadi et al., 2013). They found that monoterpene-linalool and the sesquiterpene nerolidor are the more dominant compounds in cultivated strawberries, while in wild strawberries; olefinic monoterpenes and myrtenyl acetate are more prevalent. Linalool imparts "sweet", "floral", and citrus-like" note, nerolidor imparts a "rose", "apple", and "green" note, olefinic monoterpenes contribute the "turpentine-like", "woody", "resinous", and "unpleasant odour of wild strawberry", and myrtenyl acetate imparts the typical aroma of the wild strawberry species (Summarised by Aharoni et al., 2004). Recently, Aharoni et al. (2004) identified the *F. ananassa Nerolidol Synthase1 (FaNES1)* gene in cultivated strawberry. They found *FaNES1* to be the dominantly expressed gene in ripe cultivated strawberry fruit, which has provided them with a strong selective advantage.

Similarly, Forney et al., (2000) found almost 35 fold differences among different strawberry cultivars. The abundant chemical volatiles were methyl and ethyl ester but it also depended on the cultivar (Forney et al., 2000). However, other volatiles may present in specific cultivars and give a unique aroma. It was also reported that the genetic variation could affect the accumulation of TSS and TA as these process controlled by specific genes which differ between cultivars (Crespo et al., 2010). Thus, genetic diversity can be considered as a major factor affecting

flavour quality in addition to other factors such as storage condition and/or duration, and pre-harvest or post-harvest factors.

1.4.3.2 Maturity

Aroma development is one of the most important changes taking place during fruit ripening. Volatile compounds may vary quantitatively and qualitatively depending on maturity stage as they normally increase with ripening development. Forney et al., (2000) studied the composition of volatiles among different strawberry cultivars and maturity stages and concluded that red-ripe fruits contained 5-fold greater volatiles comparing with 75 % red fruits at time of harvest. These findings are in consistent with the findings of many previous studies which concluded that production of flavour volatiles increased dramatically during ripening and the greatest production observed in fruits harvested red-ripe (Kalt et al., 1993; Miszczak et al., 1995).

Forney et al., (2000) also reported that ester, furaneol, mesifurane, and furanoel glucoside increased while fruit ripening in all cultivars (Forney et al., 2000). It was also reported by researchers studied the changes of strawberry volatiles at different maturity stages, that esters (Miszczak et al., 1995; Yamashita et al., 1977) and furanones couldn't be detected at the first ripening stages and they kept increasing during development (Ménager et al., 2004). The ability of strawberry to produce volatile compounds including esters and furanones during different ripening stages, especially at the late stages, could be explained by the conclusion

of previous researchers who found an increased activity of *alcohol acyltransferase* (AAT) during ripening development (Pérez et al., 1996; Perez et al., 1996; Yamashita et al., 1977). These results may indicate that strawberry fruits should ideally be harvested at full-red stage where they reach their optimum quality.

Non-volatile compounds including sugars were considerably varied between fruits at any pick and between harvest dates (Watson et al., 2002). It was reported that the commercial range of the TSS in strawberries is 7-12 ºBRIX depending on the genotype and maturity stage (Galletta et al., 1995). Ménager et al. (2004) reported an increasing of TSS and decreasing of TA as fruit ripened. Sucrose, fructose, and glucose levels increased 4-fold, 1.4-fold, and 1.5-fold, respectively during maturity development. However, the major organic acid reported was citric acid which accounting for up to 70-80 % of total acid content of ripe strawberry (Crespo et al., 2010; Watson et al., 2002). This acid contributes greatly to fruit titratable acidity (TA), which declines gradually during fruit development (Kafkas et al., 2007; Ménager et al., 2004). Low TSS with high TA was found in white-harvested strawberry comparing with red-harvested, but the opposite is true at full-red stage (Kalt et al., 1993; Ménager et al., 2004). These results indicate that the synthesis of sugars takes place during the ripening process and endorse the previously mentioned statement that strawberry fruits should ideally be harvested at full-red stage where they reach their optimum flavour.

1.4.3.3 Pre-harvest and post-harvest factors

Pre-harvest and post-harvest practices also play an important role in strawberry flavour profile including volatile compounds, soluble solid content and titratable acidity. Pre-harvest factors such as environmental conditions including; sunlight, water availability, and fertilization have been related to influence flavour volatile compounds (El Hadi et al., 2013). Overall, lowering light intensity led to lower content od ascorbic acid and sugar which will eventually affect the flavour of the fruit (Knee, 2002). Heavy rain, water deficiency or nitrogen deficiency were reported to minimize the tomato flavour, whereas in apple volatile production influenced by aminoethoxyvinylglycine (AVG) application (Cited by El Hadi et al., 2013). TSS content seems to be more dependent on environmental conditions. Previous work showed higher TSS content in summer-planted strawberry fruits comparing with winter-planted fruits (Watson et al., 2002).

Post-harvest factors can also affect the aroma compounds and concentrations. These include, but are not limited to, post-harvest handling, storage condition, and chemical application. Levels of fructose and glucose tend to increase, but the amount of sucrose decreased with storage (Kafkas et al., 2007) which may be due to the hydrolysis of sucrose into fructose and glucose (Fait et al., 2008). Different techniques could be used to prolong the strawberry shelf-life including heat, cold, and storage atmosphere, but they also were reported to affect the flavour (El Hadi et al., 2013).

Considerable evidence of the negative effects of low and high temperature storage has been reported on different fruit flavour including tomato (Maul et al., 2000) and strawberry (Schwieterman et al., 2014). Maul et al., (2000) stated that storing tomato at 10 °C showed better quality, higher concentration of major aroma volatiles, and higher content of TSS compared with other temperature treatments (5, 12.5, and 20 °C). Furthermore, light and high temperature storage were reported to increase the TSS content of strawberry, but without any effect on TA (Kalt et al., 1993). Miszczak et al., (1995) studied the effect post-harvest storage (temperature and light) on strawberry quality traits (volatiles, colour, FW loss, and anthocyanin content) and found that volatile compounds were temperature and light dependent. They could increase the production of volatile compounds, especially ester, by increasing the ester biosynthesis from amino acids (Miszczak et al., 1995). These findings also supported by the conclusion of Watson et al., (2002) who studied the effect of shading on the production of volatiles and concluded that the high amount of shading the fewer amount of volatiles. In contrast, Schwieterman et al., (2014) stated that increasing temperature lead to increasing the maturation rate and decrease TSS. This contradiction could be explained by the consensus of many researchers that genetic diversity can be considered as a major factor affecting flavour quality.

As a final point, still there is a need for more information that addresses how quality traits of strawberry being influenced by genotype (G), environment (E)

and their interactions (G x E). The interaction effects were previously reported between the genotype (G) and environment (E) for many quality traits (Figure 1.8), which highlights the importance of evaluating populations during several years and different cultivation sites with standardized experimental design to be able to elucidate the genetic basis of trait variation by the means of the applications of genomics in strawberry (for more details refer to the section 1.5).

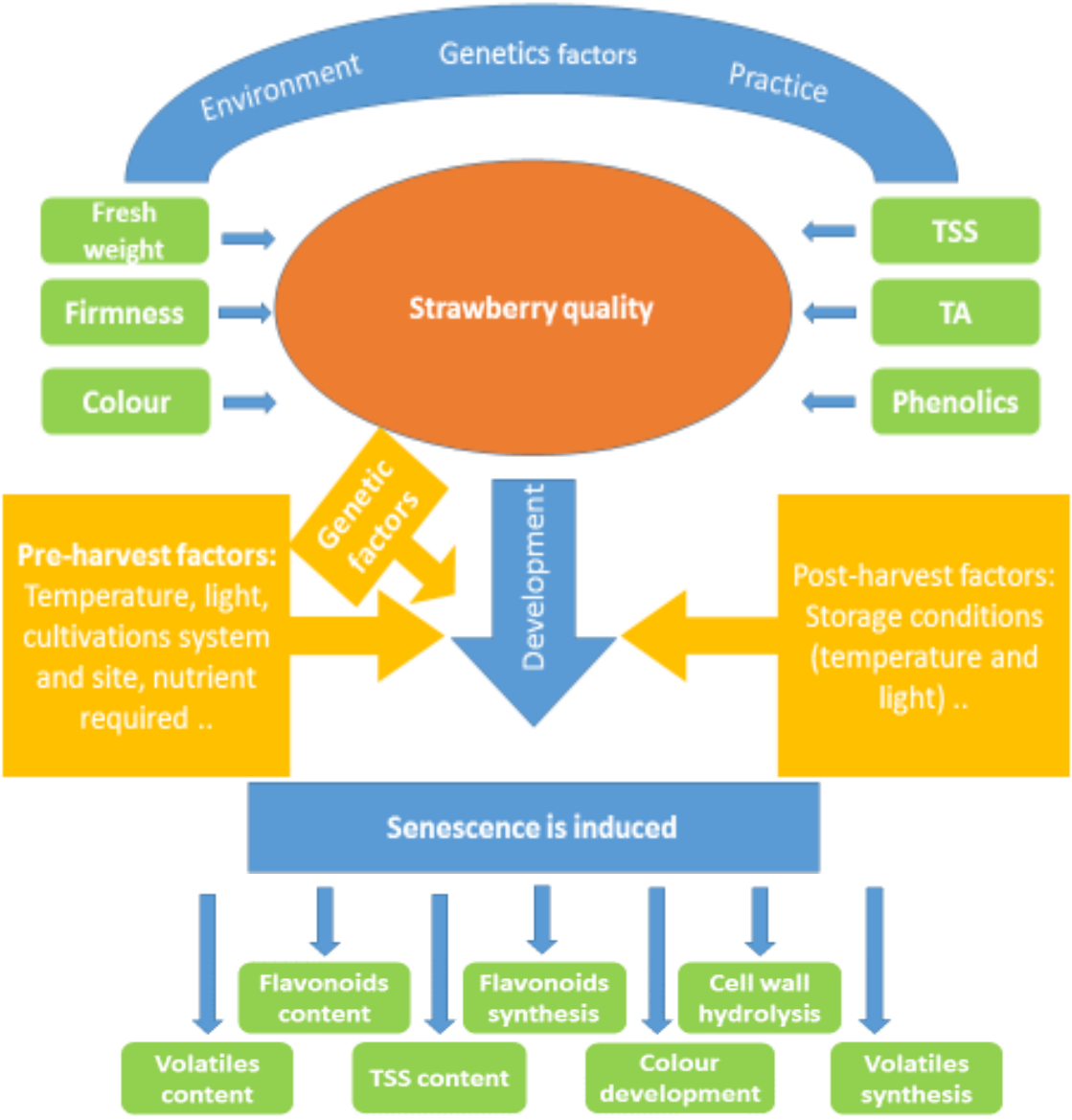


Figure 1.8. Simple model to illustrate factors (genetic and environment) affecting fruit quality of strawberry.

1.5 Applications of genomics in strawberry

Applications of genomics in cultivated strawberry have been very slow due to many factors including its highly complexity as an allo-octopliod genome, limiting of genomic resources and the high cost of such techniques. Strawberry breeding programmes have focused on obtaining new cultivars with improved fruit quality traits based on traditional breeding process (Figure 1.9). In simple words, the selection process of the parental lines takes place based on their favourable traits (Prohens, 2011). Then, the evaluation of the traits is made to know which line(s) of the offspring have the best traits before the process continue for the next generation. Although this principle has been successfully implemented, Lasley et al. (1994) have summarised up to six variables that limit the success of the traditional breeding, one of them is physical space. As a consequence of these limitations of the traditional breeding, in the 20th century a considerable number of genomic studies have targeted strawberry in order to accelerate the selection process and make it more efficient. One gene or more control many traits in cultivated strawberry. Therefore, the approaches of quantitative genetics are essential for determining the types of genetic variance that contribute to economically important traits and how selecting one trait influences another trait.

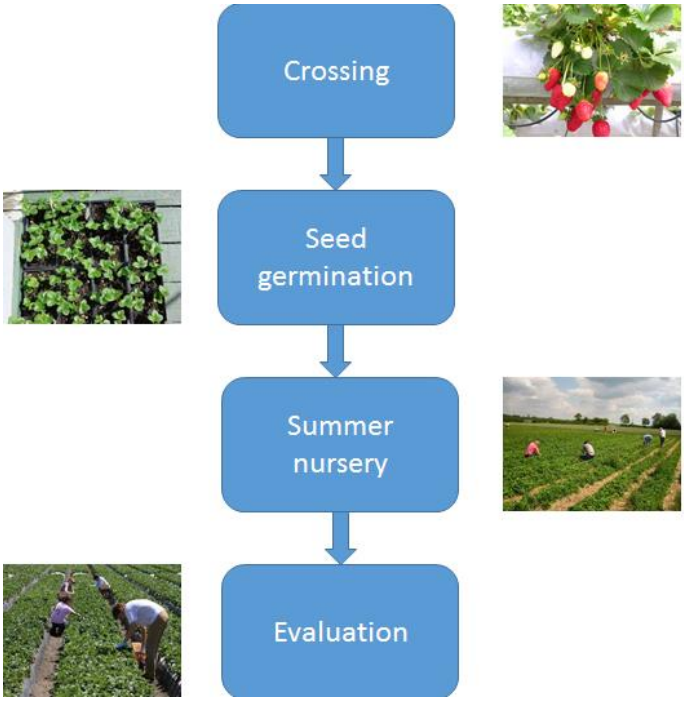


Figure 1.9. Traditional breeding process of strawberry.

The applications of genomics in strawberry can be divided into three different areas; fingerprinting for clone identification (Chambers et al., 2013), gene identification (Chambers et al., 2014), and Quantitative trait locus identification (QTL) (Lerceteau-Kohler et al., 2012; Zorrilla-Fontanesi et al., 2012, 2011). QTL are a chromosomal regions that contains a gene/genes that regulate a measurable characteristic/trait. This region must be polymorphic (have allelic variation), to have an effect in a population, and must be linked to a polymorphic marker to be detected. QTL analysis is based on a sophisticated statistical approach that helps to detect the association between the phenotype and the genotype of markers. This will help to identify the most likely genomic region(s) that is linked to or contains

gene(s) contributing toward the variation of specific trait and will help to discover more of their location, action and interaction.

The first time QTL principle was used in beans in 1923 to map a QTL for seed size (Reviewed by Swamy and Sarla, 2008). This technique has been recently used among several crops to enhance productivity and quality traits in tomato and lettuce (Causse et al., 2002; Zhang et al., 2007), yield in wild species of crop plants including rice, tomato, barely, wheat, soybean, beans, and grains (Swamy and Sarla, 2008). The most studied crops used for mapping of QTL in yield in wild species are tomato and rice (Tanksley and McCouch, 1997). In strawberry, the most impact of QTL adaption is for analysis of genetic variation, genetic mapping and cultivar identification (Whitaker, 2011).

In strawberry, studies using QTL have been limited to the physiological and molecular mechanisms of plant development and ripening. This might be due to the high complexity of allo-octoploid genome (*2n = 8x = 56*) with probable contributions from up to four diploid ancestors (Rousseau-Gueutin et al., 2009; Zorrilla-Fontanesi et al., 2011). A number of cytological genome models have been proposed for the octoploid species, but the most widely accepted to date is that of Bringhurst (1990), who proposed the genomic conformation AAA'A'BBB'B'. This assumes a diploidization of the octoploid *Fragaria* genomes and disomic inheritance (Bringhurst, 1990; Hirakawa et al., 2014; van Dijk et al., 2014).

The first genomic study on strawberry (octoploid *Fragaria*) was in 2003 where Amplified Fragment Length Polymorphism (AFLP) markers were used to construct the first linkage map for cultivated strawberry progeny from a cross between the variety Capitola and the clone CF1116 (Lerceteau-Köhler et al., 2003). Later on, Weebadde et al. (2007) developed another linkage map for octoploid strawberry from the cross of the Tribute × Honeoye which contains only AFLP and spanning genetic distance of 1541 cM. On that time, Spigler et al. (2008) also reported the first linkage map containing SSR markers (210 SSR markers) and spanning 2,373 cM, which in two years later was then saturated (Spigler et al., 2010). After that, the genetic linkage map derived from the cross Redgauntlet × Hapil containing 170 loci and 182 loci and covering 1675 cM and 1440 cM for the female and male linkage map, respectively, was reported by Sargent et al. (2009). This was then also saturated by further mapping 330 loci, resulting in a linkage map containing a total of 549 loci and spanning the genetic distance of 2,140 cM (Sargent et al., 2012). Soon after, Zorrilla-Fontanesi et al. (2012, 2011) developed a genetic linkage map derived from the cross between two octoploid strawberry selection lines (232 × 1392) which contains a total of 363 SSR markers and covers a genetic distance of 1,400 cM. Recently, a high quality integrated linkage map using SSR markers was developed for an octoploid strawberry progeny (Holiday × Korona), which contains 508 SSR loci and covered genetic distance of 2,050 (van Dijk et al., 2014). These results showed that studies using QTL in octoploid strawberry only recently have been started.

QTL and candidate genes of strawberry (*Fragaria* × ananassa) for several traits including yield, sugars, ascorbic acid, acidity, colour, firmness, day neutrality, diseases resistance, and volatile compounds were investigated, however AFLP and SSR markers were the preferred marker system (Antanaviciute et al., 2015; Lerceteau-Kohler et al., 2012; Weebadde et al., 2007; Zorrilla-Fontanesi et al., 2012). Zorrilla-Fontanesi et al., (2011) detected 33 QTL for 14 agronomical and fruit quality traits from the analysis of the interspecific cross between two different lines (232 and 1392), using genetic SSR linkage map.

Recent developments in next generation high-throughput DNA and RNA sequencing and genotyping technologies have allowed the prompt progress of high quality genetic linkage maps of various crops in the *Rosaceae* family using genotyping arrays. This development permits researchers to sequence and genotype thousands of single nucleotide polymorphism (SNP) markers in a single array. Furthermore, a first high-throughput genotyping array for octoploid *Fragaria*, the Affymetrix IStraw90® Axiom chip, described by Bassil et al. (2015) was used for genotyping Redgauntlet × Hapil mapping progeny consisting of 140 individuals by Dr Richard Harrison and his team (East Malling Research, UK; unpublished). The novel SNP-based linkage map contained a total of 3,933 unique SNPs and spanned 28 linkage groups of the octoploid strawberry, covering a genetic distance of 2,624.7 cM.

1.5.1 Principle of QTL

It is well-known that most important traits of the crop such as quality traits, agronomical traits and disease resistance forms are controlled by single gene or multiple genes which result in as quantitative traits. These traits may co-segregate with nearby marker loci, which help to identify the QTL. Therefore, QTL are normally mapped by using the markers to partition the mapping population into different genotypic classes based on the genotypes at the marker locus, and apply the correlative statistics to determine whether the individuals of one genotype differ significantly with individuals with other genotype with respect to the measured trait.

1.5.2 Steps in QTL analysis

All marker-based mapping experiments have the same basic strategy. This strategy involves five following steps:

I. Select parents that differ for a trait.

II. Generate recombinant inbred lines (can be F1, depends on the population).

III. Genotyping; screen the two parents to identify polymorphic marker loci and develop the genetic map by screening all the progeny of the cross.Phenotyping; screen in field or in protected conditions for the traits of interest.

IV. Conduct a correlation analysis between the phenotypic and the genotypic data in order to identify the QTL of interest.

By using molecular markers such as single nucleotide polymorphism (SNP), restriction fragment length polymorphism (RFLPs), Random Amplified Polymorphic DNA (RADP), Amplified Fragment Length Polymorphism (AFLP), Simple sequence repeats (SSR), the QTL process has become simpler and quicker (Miles and Wayne, 2008; Swamy and Sarla, 2008). SNPs and SSR markers are preferable for QTL mapping as they have the ability to identify the homozygotes and heterozygotes in a segregating population (Swamy and Sarla, 2008). As soon as the genetic markers that linked to a QTL that control the trait of interest have been identified and validated, we could use these to select the lines that have the desirable QTL for future breeding. Certainly, the estimation of QTL locations on a linkage map is still needed to bridge from markers to candidate gene models that then provide information on linked markers that can be used during marker-assisted selection in breeding programmes, making the selection process more efficient.

1.5.3 Application of QTL mapping

The introgression of QTL into elite lines and marker assisted selection (MAS) had been used in many crops including maize, tomato, rice and wheat (Prasanna, 2003; Veeresha et al., 2011). For plant breeders, the precise location of the QTL may not be that important as the QTL has a large effect and can be introgressed using marker assisted backcrossing.

1.6 **Summary**

Post-harvest quality traits, such as colour, firmness, flavour (TSS and TA), and phenolic content, are becoming very important traits for breeders and consumers. Phenolic content, which is linked with the ability to protect human health against many diseases, could be influenced by different factors such as genotype, pre-harvest and post-harvest factors. Similarly, post-harvest quality traits including FW, TSS, TA, firmness, colour and aroma volatiles could be influenced by the same factors. Thus, there is a need in breeding programmes to maintain and enhance these traits in the fruit to increase consumption and make the fruit appeal to the widest possible range of consumers. Maintaining these traits is possible by either manipulating pre- and/or post-harvest factors, however breeding programmes still in a need to understand the genetic mechanism to control each trait in order to develop them. This solution could be achieved by the use of linkage maps together with the phenotypic characterization of these traits in order to allow identifying the candidate position in the genome. The identification of these positions (QTL) and the development of markers linked to the traits of interest will enable plant breeders to use marker-assisted selection (MAS) breeding in the future. Thus, a QTL approach is more powerful than just looking at genes thought to be involved in biosynthesis as it enables a number of different control points for each trait of interest to be identified.

1.7 Study aims and objectives

The cultivated strawberry (*Fragaria* × *ananassa*) is an economically important soft fruit with a complex octoploid genome. However, sizeable numbers of genetic markers for strawberry breeding purposes have only recently been developed. The aim of this study was to characterise the variation in quality traits among the F1 population developed from the cross of Redgauntlet x Hapil (RG x H) (Sargent et al., 2009), and to detect QTL linked to key postharvest quality traits. These traits include total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio, fresh weight, colour parameters, firmness, and phenolic compounds.

The population used in this project was derived from a progeny of 188 seedlings from the cross RG x H, a heterozygous cross that segregates for fruit quality, disease resistance and postharvest traits (Sargent et al., 2009). Because the strawberry is a highly heterozygous species, an F1 population with a two-way pseudo-testcross was used to create genetic linkage maps (Grattapaglia and Sederoff, 1994). Mapping of QTL can lead to a better understanding of the associations between phenotype and genotype, how quality is regulated at the genetic level and how different traits are genetically correlated. Hence, this study could provide the basis for future academic work in identifying and isolating the regulatory genes linked with these traits and a fundamental understanding of their genetic controls, which in turn could facilitate molecular marker development

through the usage of marker assisted selection (MAS) or genomic selection (GS) methods.

For this study, the objectives are as follows:

- To assess post-harvest measures of quality (total sugar content [TSS], titratable acidity [TA], fresh weight, colour parameters, firmness, and phenolic compounds) with respect to storage, different sites and varying environments (Chapter 3).
- To assess the segregation of the RG x H population for quality traits and to identify the QTL linked to those traits (Chapter 4).
- To evaluate and characterise the flavour profile (i.e. volatile and non-volatile compounds, including TSS and TA) linked to flavour perception and to identify any correlations between sensory and instrumental analysis (Chapter 5).

Chapter 2 : General materials and methods

2.1 Introduction

This chapter describes the materials and methods used for the experiments reported in the thesis.

2.2 Reagents and standards

2.2.1 HPLC

Polyphenol standards were supplied as follows: Ellagic acid, (+)-Catechin, Kaempferol, Quercetin, Pelargonidin chloride and Cyanidin chloride by Sigma (North Dorset, UK), the spectrum of the polyphenols' standards and calibration curves are shown in the appendix, sections 3.1 & 3.2.

HPLC-grade methanol and water were purchased from J. T. Baker (Deventer, the Netherlands). Formic acid was obtained from Merck (Darmstadt, Germany). The glassware was cleaned before use by repeatedly washing with a hot mixture of chromic and concentrated sulfuric acid and rinsed with purified water and finally dried at 150° C.

2.2.2 GC

3-Heptanol, used as internal standard, and Calcium chloride ($CaCl_2$) were both purchased by Sigma–Aldrich (Gillingham, UK).

2.3 Plant growth and material

The mapping progeny of Sargent et al. (2009) consists of a full sib family of 173 individuals generated from a cross between the two strawberry cultivars 'Redgauntlet' and 'Hapil'. Crosses were performed and seedlings were germinated and grown according to the method described by (Sargent et al., 2009). This progeny segregates for fruit quality, disease resistance and postharvest characteristics (Sargent et al., 2009). The parents had been chosen because they differ in important quality traits; Hapil has large fruit size with a sweet taste, whereas Redgauntlet (RG) has small fruit with a bland taste. They also differ in flowering time where Hapil (H) classified as a mid-season type (June-bearers), whereas RG is slightly later season type (June-bearers).

The trials were carried out in two different sites characterised by different conditions: at East Malling Research (EMR) (New Road, East Malling, Kent) in an open field system and at University of Reading, Whiteknights campus (Reading, Berkshire, UK) in a glasshouse system. Details of the respective latitude, longitude, elevation, and temperature are given in Table 2.1.

Table 2.1. Geographical and climatic conditions at two different sites during the strawberry seasons, April - June 2013 for EMR, April - June 2014 for Reading.

	EMR (2013)	Reading (2014)
Latitude	51° 17' 13"N	51° 26' 26"N
Longitude	0° 27' 0"N	0° 56' 11"N
Elevation (meter)	33.0	66.0
Average temperature (°C)	11.1	13.0
Standard deviation of temperature (°C)	±3.9	±3.0
Maximum temperature (°C)	25.2	24.4
Minimum temperature (°C)	−4.5	−0.4

2.3.1 Experiment of 1st year (2013-2014)

Strawberry plants of the F1 mapping population were raised in the glasshouse from a cross between the two octoploid strawberry (*Fragaria x ananassa*) cultivars Redgauntlet (RG) and Hapil (H) at EMR (New Road, East Malling, Kent). The cultivation was conducted as following (Antanaviciute, 2016): 188 seedlings were raised from the cross and of those 120 seedlings were randomly selected and further clonally propagated twice (during summer 2012 and during summer 2014) by pinning down the runners of the mother plants.

2.3.1.1 Experimental design

Six replicates of the 122 seedlings and parental lines ‘Redgauntlet’ and ‘Hapil’ were produced, with a total of 732 plants (including parents), were planted in an open field system at EMR in late September 2012 and mid-August 2014 (the randomisation plan is shown in the appendix, sections 2.1). Seedlings were randomly distributed within three tunnels/blocks, where each tunnel/block had

three beds and two rows per bed (Figure 2.1). Seedlings were planted in a double row in zig-zag (40 cm between plants) on raised beds, 35 cm high and 50 cm wide.

Figure 2.1. Seedlings of the 'Redgauntlet' × 'Hapil' mapping population and parental genotypes planted in the field at EMR**;** a) seedlings without cover before phenotyping, Photograph was taken on 17.05.2013; b) seedlings under cover while collecting phenotypic data, Photograph was taken on 25.06.2013; c-e) seedlings in each tunnel/block, Photographs were taken on 12.08.2013.

Plants in the field trial were allowed to grow and establish naturally over winter. All runners and dead material was removed in spring for ease of phenotyping. The field trial plots were covered with polyethylene while plant phenotyping was on-going; this was later (late July) removed in order to avoid disease (Figure 2.1). An irrigation system was installed in each row, and plants were watered and fertilized following conventional practices and depending on weather conditions. Plants were sprayed against common pests (aphid), insects (spotted wing drosophila) and diseases (mildew and botrytis) before, during and after the phenotyping season.

The spraying programme for the season was as follows: once a week for 23 weeks for mildew (March - September), once a week for ten weeks for Botrytis (May - September), a single spray for spotted wing drosophila (in August) and five sprays for aphid (March - June).

Fully-ripe fruits by the commercial standard (90-100 % red) were harvested from two blocks and delivered immediately to the laboratory in the School of Chemistry, Food and Pharmacy, University of Reading, Whiteknights campus (Reading, Berkshire, UK) at ambient temperature. Strawberries were harvested by picking all ripe fruits twice in 2013. Two fruits of each genotype were harvested from two blocks, which represent four biological replicates (n=4) at each time point (Table 2.2). Fruits were placed into clear plastic egg boxes to avoid bruising and to allow the analysis of individual fruit then stored at a commercially relevant temperature of 4 °C in the dark overnight before analysis of fresh weight and colour, using non-destructive methods enabling repeat measures of the same fruits, as well as sample preparation for later analysis including TSS, TA, and phenolic content, using destructive methods, at two post-harvest days (day 1 and 7). The storage temperature used in this experiment was 4^{o}C which was considered within the optimum temperature recommended for maintaining postharvest quality in strawberries for a week (0 ± 5 °C) (Ayala-Zavala et al., 2004). Only two postharvest time points were possible because of the limited number of harvested fruits.

Table 2.2. Sample size (n) for the experiment of 1st year.

Trait	n	Trait	n
FW	4	TSS	4
Colour	4	TA	4
Firmness	4	Polyphenols	2

Post-harvest quality assessment was conducted on fresh fruits including FW, colour and firmness. Then, one experimental rep of each block was prepared, by blending the two fruits, which were stored at -80 °C prior to further chemical analysis. Immediately on the day of HPLC analysis each experimental rep was measured twice (two technical reps) for TSS and TA. Then one sample of each experimental rep was extracted for HPLC analysis; more details for each measurement are described in Table 2.2.

2.3.2 Experiment of 2nd year (2014-2015)

In the autumn on 2013, approximately 140 genotypes including the parents, each represented by two stock plants, were propagated in 3.5" square pots at University of Reading, Whiteknights campus (Reading, Berkshire, UK) for the second year experiment (2014-2015). The offspring were grown in a polytunnel over the winter to accumulate the required vernalisation. All plants were watered and fed as needed. Feeding through irrigation system was conducted using NPK Sangral Soluble Fertiliser (1:1:1). It is readily soluble in water to provide instantly available nutrients for root and foliar uptake.

In the spring, strawberry plants were planted in 0.5 metre peat-based grow bags (Bulrush Horticulture Ltd., UK) in two randomized blocks in an experimental glasshouse at University of Reading, Whiteknights campus (Reading, Berkshire, UK) (Figure 2.2). The glasshouse was set to heat at 5 °C and vent at 20 °C. The plants were grown in natural light. Plants were kept well-watered and well-fed by using a drip irrigation system with three drippers per bag. The feed composition consisted of calcium nitrate, potassium nitrate, potassium sulphate, magnesium nitrate, monopotassiumphosphate, iron-EDTA, manganese sulphate, copper sulphate, zinc sulphate, sodium molybdate, and solubor. The recipe was invented according to the commercially grown strawberry plots and been also applied on another strawberry study at University of Reading. Chemical treatments for powdery mildew, botrytis, and aphids were applied as necessary.

Figure 2.2. Seedlings of the 'Redgauntlet' × 'Hapil' mapping population and parental genotypes planted in the glasshouse at Reading. a) the glasshouse from outside, Photograph was taken on 05.04.2014; b) seedlings in a bed, where each block has 4 beds, Photograph was taken on 07.05.2014; c) flower initiation stage, Photograph was taken on 08.05.2014; d) seedlings in a block, where 2 beds are shown, Photograph was taken on 08.04.2014.

2.3.2.1 Experimental design

Random block experimental design was used in this experiment. The experimental design consisted of two blocks; each block had 140 genotypes, of which each had 2 replicates, giving a total of 560 plants (the randomisation plan is shown in the appendix, sections 2.2). Each block had four beds and three rows per bed, where five plants were established in each bag with 56 bags for each block.

Fully-ripe fruits, as prescribed in section 2.3.1.1, were harvested from the two blocks and delivered immediately to the laboratory at ambient temperature. Strawberries were harvested by picking all ripe fruits once a week for four weeks. Three fruits of each genotype were harvested from two blocks, which represented six biological replicates (n=6), and were placed into clear plastic egg boxes to avoid bruising and to allow the analysis of individual fruit and then put in cold store (4 °C) overnight. Post-harvest quality assessment was conducted on fresh fruits including FW and colour using non-destructive methods allowing repeat measurements of the same fruit (Table 2.3). Then, one experimental rep of each block was prepared after measuring the firmness, by blending the three fruits used for firmness measurement, and was stored at -80 °C for further chemical analysis. Immediately on the day of HPLC analysis each experimental rep was measured twice (two technical rep) for TSS and TA. Then one sample of each experimental rep was extracted for HPLC analysis; more details for each measurement are described in table 2.3.

Table 2.3. Sample size (n) for the experiment of 2nd year.

Trait	**n**	**Trait**	**n**
FW	6	TSS	4
Colour	6	TA	4
Firmness	6	Polyphenols	2

2.3.3 Experiment of 3rd year (2015-2016)

In the autumn of 2014, eight genotypes plus the parental lines, each represented by two stock plants, were propagated in 3.5" square pots at University of Reading for the 3rd year experiment (quality assessment for extreme lines of the population including sensory analysis "2015-2016"). As the main target was sensory/flavour analysis, the selection was based on sugar and acid content (TSS, TA, and TSS/TA ratio; Selection protocol for F1 progeny individuals shown in the appendix; section 5.1). Extreme lines of sugars and/or acids content were selected, so that the taste was likely to be distinctive enough to show differences. The daughter plants were grown in a polytunnel over the winter to accumulate the required vernalisation. All plants were watered and fed, with the same nutrient recipe mentioned in section 2.3.2, as needed. Feeding through irrigation system was conducted using NPK Sangral Soluble Fertiliser (1:1:1).

In the spring, strawberry plants were planted in 0.5 metre peat-based grow bags (Bulrush Horticulture Ltd., UK) in three randomized blocks in an experimental glasshouse at University of Reading. The glasshouse was set to heat at 5 °C and vent at 20 °C. The plants were grown in natural light. Plants were kept well-watered and well-fed by using a drip irrigation system with three drippers per bag (the feed composition is prescribed previously in section 2.3.2). Chemical treatments for powdery mildew, botrytis, and aphids were applied as necessary.

2.3.3.1 Experimental design

Random block experimental design was used in this experiment. The experimental design consisted of three blocks; each block had 10 genotypes, of which each had 10 replicates, giving a total of 300 plants (the randomisation plan is shown in the appendix, sections 2.3). Each block had two beds and three rows per bed, where three plants were established in each bag (except one bag with 4 plants) with 33 bags for each block.

Strawberries were harvested at commercial ripeness from the three blocks block by picking all ripe fruits twice a week for four weeks. Twenty-seven fruits of each genotype were harvested from each block which then divided into 9 fruits for physicochemical traits, 12 fruits for sensory, and 6 fruits for volatile compounds detection per shelf life day (Table 2.4). Fruits were placed into clear plastic egg boxes to avoid bruising and to allow the analysis of individual fruit and then put in cold store (4 °C) overnight.

Table 2.4. Sample size (n) for the experiment of 3rd year.

Trait	n	Trait	n
FW	9	TSS	3
Colour	9	TA	3
Firmness	9	Polyphenols	3
GC	3	Sensory analysis	6

2.4 Harvest

Ripe fruits of each genotype from all blocks were harvested into punnets, and then taken to the laboratory for quality assessment. Only fully coloured developed fruit without defects were selected on a visual basis. Punnets were stored in cold store (4 °C) for overnight and then analysed at three postharvest points, starting from day 1, depending on the fruits maturity/availability.

2.5 Assessment of postharvest fruit quality and QTL detection (1st & 2nd year experiments)

For each genotype (parental and F1 progeny lines), a total of six quality traits were monitored on different post-harvest days (day 1, day 4 and day 7) during two successive years (2013 and 2014).

2.5.1 Fresh weight

Fresh weight of samples was measured on day 1, day 4 and day 7, depending on the fruits availability, to evaluate the water loss from the fruits using a digital electrical balance (Analytical products LTD, England).

2.5.2 Colour measurement

Three measurements were taken on day 1, day 4 and day 7, depending on the fruits availability, using a sph850 spectrophotometer (ColorLite GmbH, Katlenburg-Lindau, Germany) around the circumference of each fruit and a single mean set of values was calculated from three replicate measurements of each fruit. The

instruments included three parameters L* (luminescence), a* (red tone), b* (yellow tone) (Figure 2.3).

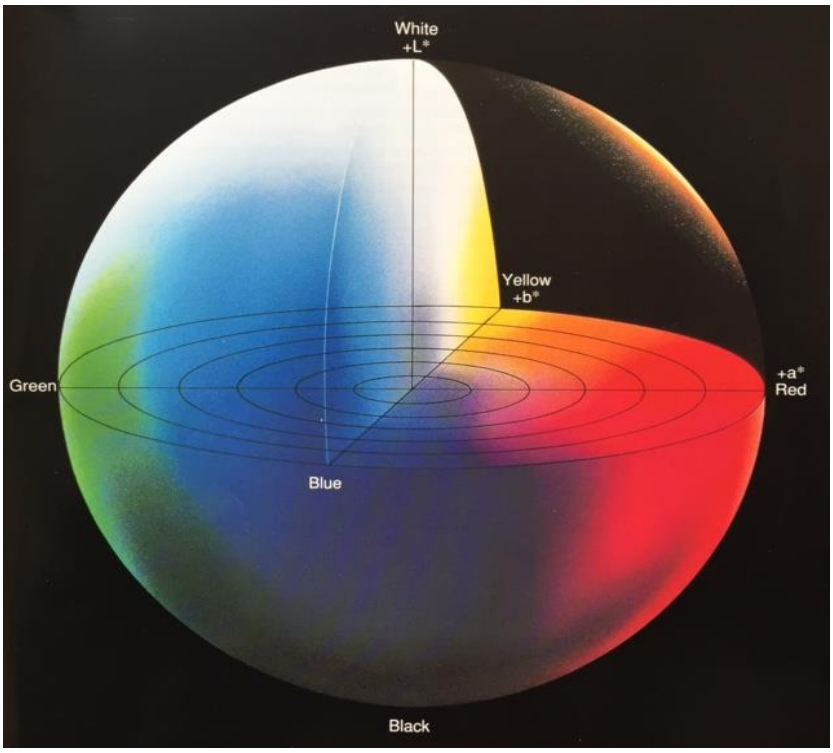


Figure 2.3. CIE L*a*b* colour space. L* indicates lightness, a* and b* are XY colour coordinates indicating colour directions; a* is the red–green axis, b* is the yellow and blue axis; the centre is achromatic grey. Adapted from Minolta (1998) with permission.

2.5.3 Firmness

Three measurements were taken on day 1, day 4 and day 7, depending on the fruits availability, using Handheld Digital Fruit and Vegetable Ripeness/Hardness Tester fitted with 3.5 mm diameter plunger tip (HFH81, Omega Engineering Limited, Manchester, UK) of each fruit and a single mean set of values was

calculated. Three measurements were taken per fruit and then averaged for each fruit. A puncture test was performed on the fruit cheek, approximately between the calyx and blossom end, by holding the fruit against a hard surface before forcing the plunger tip into the fruit at a uniform speed so that the depth of penetration was consistently to the subscribed line on the tip.

2.5.4 Sample preparation for further analysis

Selected fruits were sliced, blended and stored at -80° C on day 1, day 4 and day 7, depending on the fruit availability, for further chemical analysis:

I. Total Soluble Solids (TSS).
II. Titratable Acidity (TA).
III. Phenolic compounds analysis (HPLC).

2.5.4.1 Total Soluble Solids (TSS)

TSS is a refractometric index that indicates the proportion (%) of dissolved solids in a solution (Beckles, 2012). The TSS was determined for day 1, day 4 and day 7, depending on the fruit availability, samples through a digital, hand held refractometer (Atago, Japan). A drop of strawberry puree was placed on the hand refractometer with results expressed as °Brix.

2.5.4.2 Titratable Acidity (TA)

TA was quantified for day 1, day 4 and day 7, depending on the fruit availability, in samples by diluting each 3 ml of strawberry liquid in 50 ml distilled water and

then titrate with NaOH (0.1M), prepared by dissolving 4 g NaOH in 1000 ml distilled water, using phenolphthalein (ph-th) (0.1 %) as an indicator. Ph-th was made up as a 0.1 % solution in ethanol:water mixture (50:50 v/v). Results were converted to percent citric acid using the following equation: [(ml NaOH × 0.1N × 0.064 / 3 ml of strawberry puree) × 100].

2.5.4.3 Extraction of Flavonoids and Acid Hydrolysis for HPLC

Samples for determination of phenolic content by HPLC were extracted as follows: 3 strawberry samples of each genotype were blended together with no further addition of liquid. 1 g of strawberry puree was added to 1 ml of 70 % MeOH, prepared by mixing 70 ml of absolute methanol (MeOH) and 30 ml of distilled water. 1000 µl from the solution was transferred to screw-cap tubes and then placed in a water-bath (80 °C) for 10 minutes. Afterwards, the samples were recovered and then located in speed vacuum (Savant Speed Vac, Thermo Scientific, MA, USA) for 60 minutes to dry them completely. 1.5 ml of 2M hydrochloric acid (HCl) in HPLC grade MeOH, which was made by mixing 16.8 ml of 37 % HCl and 83.2 ml of HPLC grade MeOH, was added to each sample. Then, the samples were placed in the roller/stirrer for 45 minutes and covered with aluminium foil to prevent the degradation of light-sensitive compounds including flavonoids. After mixing, these samples were located in a dried heater block (85 °C) for 60 minutes, in order to accelerate the acid hydrolysis process, and then left for 30 minutes to cool. After that, the samples were transferred into

microcentrifuge tubes (1.5 ml) after filtering them through 0.45 µm filters. Lastly, 100 µl was transferred into amber glass vials and analysed by high performance liquid chromatography (HPLC).

2.5.4.4 High Performance Liquid Chromatography (HPLC)

The different components were separated using a Hewlett Packard (Agilent, Bracknell, UK) model 1100 series LC running HP ChemStation software with a Nova Pak C18 column (250 * 4.6; 4 mm) (Waters, Elstree, UK) at 30 °C. 50µl of each sample was injected into the column. The mobile phase consisted of (A) H_2O (95 %), methanol (5 %), and containing formic acid (0.1 %) and (B) H_2O (50 %), acetonitrile (50 %), and containing formic acid (0.1 %), with a flow rate of 0.7 ml/min. The gradient system was used as according to Table 2.5. A diode-array detector was used to record the absorbance at the following wavelengths: 254, 280, 320, 365, and 520 nm. Flavonoids were identified by matching their retention times and UV diode array spectra with those of standards.

Table 2.5. Buffer conditions and gradient for HPLC.

Time (minutes)	A%	B%
0	95	5
5	95	5
40	50	50
55	0	100
59.9	0	100
60	95	5

2.5.5 QTL

Strawberry is a highly heterozygous species, therefore F1 population and a two-way pseudo-testcross were used to generate a genetic linkage map (Grattapaglia and Sederoff, 1994), which was generated by EMR (New Road, East Malling, Kent).

2.5.5.1 Linkage construction and QTL analysis

The first high-throughput genotyping array for octoploid *Fragaria*, the Affymetrix IStraw90® Axiom array described by Bassil et al., (2015), was used for genotyping the 'Redgauntlet' × 'Hapil' mapping progeny consisting of 140 individuals. The novel SNP-based linkage map contained a total of 3933 unique SNPs distributed over 28 linkage groups, in order to show colinearity with the diploid genome (Rousseau-Gueutin et al., 2008). This map spanned a total distance of 2,624.7 cM. The 28 LGs of the octoploid linkage map were assigned to one of the seven homoeologous group (HGs) expected in *Fragaria* (x = 7) (Rousseau-Gueutin et al., 2008). The construction was conducted as follows by Dr Richard Harrison and his team at EMR:

An F1 mapping population of 188 individuals obtained from a cross between 'Redgauntlet' and 'Hapil' was planted at EMR (New Road, East Malling, Kent). Due to propagation errors, 15 seedlings (rogues) were excluded from the analysis. Therefore, a total of 173 seedlings remained. DNA was isolated from young and healthy leaf tissue of these individuals and the parental genotypes using the

DNeasy plant miniprep kit (Qiagen) according to the manufacturer's handbook. The concentration and purity of DNA was measured using a NanoDrop ND-1000 spectrophotometer (Thermo-Scientific, Loughborough, UK). All DNA samples were diluted to ~3 ng/µl for use in PCR (polymerase chain reaction). A total of 140 seedlings and parental genotypes were selected out of 173 RGxH individuals for genotyping using 90 K Affymetrix Axiom® SNP array. The DNAs were further purified for the samples which had concentrations 10 ng/µl or lower and the absorbance at 260 nm (A260) and at 280 nm (A280) rates were lower than 1.65, before sending DNAs for genotyping.

2.5.5.2 SNP-base map construction

The consensus SNP-based genetic linkage map was constructed using perl scripts developed by Dr Richard Harrison (EMR), due to the large data set generated. Once SNP markers were assigned to each of 28 linkage groups using the perl scripting language, data was further analysed using JoinMap 4.0 (Kyazma, NL) software. Linkage groups were identified and named accordingly by comparing each chromosome to the previously reported high density SNP-based linkage map constructed for an octoploid strawberry mapping population 'Holiday' × 'Korona' (van Dijk et al. 2014).

Prior to the QTL analysis, the number of SNPs had to be reduced to 523 SNPs that covered all the 28 LGs, due to the computational limitation of MapQTL programme as it cannot function if too many markers are presented to it (Table

2.6). The total genome size was 2626 centimorgan (cM) and the average interval is 5 cM between two markers (Table 2.7).

Table 2.6. Marker selection process for mapping.

Criteria	SNPs
Initial number of SNPs	3933
Remove heterozygous SNPs (hhxhk)	-
Remove SNPs markers with segregation distortion	-
Remove SNPs at same position	2058
Reduce SNP number per chromosome to ~1 per 5 cM interval	523

Table 2.7. Summary of linkage groups, number of markers and marker interval.

Linkage group	No. of SNPs	Length (cM)	Markers interval (cM)
1A	23	86.861	3.8
1B	23	80.935	3.5
1C	21	80.591	3.8
1D	14	65.268	4.7
2A	21	162.823	7.8
2B	18	88.275	4.9
2C	19	85.982	4.5
2D	16	81.158	5.1
3A	20	116.93	5.8
3B	18	71.312	4.0
3C	20	104.909	5.2
3D	20	83.562	4.2
4A	19	72.743	3.8
4B	17	95.334	5.6
4C	16	71.627	4.5
4D	17	89.662	5.3
5A	20	113.308	5.7
5B	15	93.519	6.2
5C	19	85.913	4.5

Linkage group	No. of SNPs	Length (cM)	Markers interval (cM)
5D	18	58.348	3.2
6A	22	145.061	6.6
6B	18	107.983	6.0
6C	17	118.661	7.0
6D	19	120.831	6.4
7A	17	112.305	6.6
7B	19	86.685	4.6
7C	16	76.779	4.8
7D	21	68.624	3.3
Average LG length			93.78
Total length			2625.98
Average interval			5.0

2.5.5.3 Field screening

The RGxH F1 population consisting of a full sib family of 140 lines, together with the parents, was used to phenotype the strawberry mapping population for this experiment. Field screening was conducted over two consecutive years; 63 and 76 lines were phenotyped for year 2013 and 2014, respectively. Mean values generated by ANOVA were used for QTL detection. As the original data exhibited non-normal distribution, alternative action have been taken to normalise the distribution of the data by the log-transformation of the data using the excel function before analysis.

2.5.6 Statistical analysis (1st & 2nd year experiments)

2.5.6.1 Analysis of Variance (ANOVA)

Different modules of statistical software were employed to analyse the phenotypic data. The data obtained was statistically analysed using GenStat for windows release 16 (VSN International Ltd., Hemel Hempstead, UK). For each experiment, analysis of Variance technique (ANOVA) were carried out to test the significance of differences between shelf life, cultivation sites and genotype. Each trait was analysed by day and for differences across days for all lines and between lines to identify main effects due to genotype.

2.5.6.2 Correlation analysis

Pearson's correlation analyses were conducted using SPSS for windows release 21 among traits and with each trait between all measures of post-harvest traits over two years to highlight where correlation between traits was present. The transformed data of the quality traits was used and correlation was significant at the 0.01 level (see section 4.3.2).

2.5.6.3 QTL analysis

QTL analyses was performed separately for each year using MapQTL 6.0 (Van Ooijen, 2009). Two QTL detection methods were employed, the Interval Mapping (IM) and the Multiple QTL Mapping (MQM) followed by restricted multiple QTL model mapping (rMQM) tools (Van Ooijen, 2006). Interval mapping (IM) was conducted to initially detect QTLs in quantitative data and nearby loci with the

highest logarithm of odds (LOD) scores were selected as co-factors. Markers associated at $P<0.05$ after automatic cofactor selection were then used for multiple QTL model (MQM) computation to control the genetic background for a better position of QTLs. If the LOD value selected as a cofactor fell down the LOD threshold, the cofactor was removed and then the process was repeated until the selected cofactors remained stable (LOD profile example shown in the appendix, section 4.3). The LOD threshold of 3.2 (Van Ooijen, 1999) was used to identify potential QTLs. The graphical representation of the linkage maps and QTL were prepared using MapChart®2.2 software (Voorrips, 2002) as shown in the appendix; section 4.2). The heritability was calculated as the ratio of additive genetic variance (Vg) to total phenotypic variance (Vt), (Vt = Vg + Ve) (El-Soda et al., 2014; Wray and Visscher, 2008). Vg is the genetic variation (Vg), i.e., variance between the average values of all lines, where Ve is the environmental variation, i.e., variance between the replications of all lines.

2.6 Flavour profiles of nine extreme lines from strawberry population of RGxH progeny (3rd year experiment)

As the aim of the 3rd year experiment was flavour analysis, so the selection of the nine lines/genotypes was based on sugar and acid content (TSS, TA, and TSS/TA ratio). Fruits had different TSS and TA were selected, so that the taste is likely to be distinctive enough to show differences in sensory attributes (for more details refer to the "selection protocol for F1 progeny individuals" in the appendix;

section 5.1). All physicochemical traits including FW, colour, firmness, TSS, TA, and phenolic compounds were done according to the previously described procedures. The physicochemical (qualitative) and sensorial traits were measured on day 1 and 5.

2.6.1 Flavour assessment

2.6.1.1 Solid-phase micro extraction (SPME) for volatile compounds

Three biological replicates were prepared as follows: strawberry samples were removed from the freezer (-80° C) and 5 g was weighed out. Saturated calcium chloride (5g), prepared by dissolving 111 g of $CaCl_2$ in 150 ml distilled water, was added to the strawberries to stop the enzyme reaction which were then blended for one minute using an electric blender. Five grams of the mixture were transferred into an SPME vial (15mL) fitted with screw cap and internal standard (25 µl of 50 ppm 3-heptanol) was added to the vial. The extraction of volatile compounds was performed using a headspace solid-phase microextraction system (HS-SPME) using a 50/30 µm divinylbenzene (DVB)/polydimethylsiloxane (PDMS) fibre (Supelco, Bellefonte, Pennsylvania, USA). After equilibration at 35 °C for 10 min, the fibre was exposed to the headspace above the sample for 30 min.

2.6.1.2 GC-MS analysis of SPME extracts

The SPME fibre was inserted into the injection port of an Agilent 7890A gas chromatography system coupled to an Agilent 5975C detection system equipped

with an automated injection system (CTC-CombiPAL). The volatiles were desorbed onto a capillary column ZB-5MSi (30 m × 250 μm × 1 μm film thickness) (Phenomenex). The temperature programme used was: 5 min at 40 °C isothermal and an increase of 4 °C/min to 260 °C. Helium was used at 2.1 mL/min as carrier gas. The temperature of injector, interface and detector was 250 °C. The sample injection mode was splitless. Mass spectra were measured in electron ionization mode with ionization energy of 70 eV, the scan range from 20 to 280 *m*/*z* and the scan rate of 5.3 scans/s. With regards to data processing, the data were controlled and stored by the HP G1034C Chemstation system. Volatile compounds were identified by comparison of each mass spectrum with spectra from authentic compounds analysed in our laboratory, spectra from the NIST/EPA/NIH Mass Spectral database (2011) or spectra published elsewhere. To confirm the identification, the linear retention index (LRI) was calculated for each volatile using the retention times of a homologous series of C_6-C_{20} *n*-alkanes. The approximate quantification of volatiles was calculated from GC peak areas, by comparing with the peak area of the 3-heptanol standard, using a response factor of 1.

2.6.2 Sensory analysis

The sensory study took place at the sensory booths at The University of Reading, with neutral odour, artificial daylight, and controlled temperature. The sensory profile of the samples was generated by a trained panel of experts (ten panellists)

who agreed to use 31 terms for the quantitative assessment of the samples (Table 2.8), sensory scoring sheet shown in the appendix, section 5.2. The panellists were selected and trained in accordance with ISO standards for sensory analysis (ISO 8586:2012) and are subject to performance monitoring (ISO 11132:2012). All panellists had a minimum of 6 months' experience in sensory evaluation, and some up to eight years of experience.

2.6.2.1 Trained sensory panel vocabulary development

A list of sensory vocabulary terms for strawberry puree were established using an expert panel of ten sensory assessors (see Table 2.8 for the list of terms). This was achieved through presentation of samples in a random, coded fashion over the course of three, 30 min sessions on consecutive days. Assessors discussed, with the aid of a facilitator, the various sensory attributes associated with the odour, mouth sensation, taste, flavour and aftereffects of puree samples (definitions of agreed vocabulary terms are shown in the appendix, section 5.3). Reference standards were used where appropriate to ensure agreement of the descriptive terms chosen. Once a consensus set of descriptors was established, a formal sensory assessment was conducted.

Table 2.8. List of terms for sensory attributes associated with strawberry puree samples over shelf life days

Attribute	Agreed definition
Odour	Sweet (candy, sweet)
	Fermented (Lactic acid)
	Zesty (Fresh, citrus)
	Red berry fruit
	Green (Green strawberry)
	Ripeness
	Rubbery
	Off note
Taste	Sweet
	Acid
	Bitter
	Metallic
	Savoury
Flavour	Overall strength of flavour
	Red berry fruit
	Green (Green strawberry and leafy)
	Green (Kiwi and aromatic)
	Ripeness
	Floral (perfume, rosey)
	Cardboard (stale)
	Woody
Mouth sensation	Fizzy
	Mouthdrying
After effects	Length of finish
	Acid
	Savoury
	Cardboard (stale)
	Metallic
	Astringent
	Mouthdrying
	Salivating

2.6.2.2 Sensory rating phase

Evaluation sessions were carried out under artificial daylight conditions in an air-conditioned room (22°C), in isolated sensory booths within the Sensory Science Centre (Department of Food and Nutritional Sciences, University of Reading, UK), each equipped with computer screen, keyboard and a mouse. Compusense® five software was used to acquire the sensory data. For each sample, 6 fruits cut in halves were homogenised in a blender. A volume of 10 ml (two-three teaspoons) of the puree was introduced to the panellists in clear polypropylene tasting cups (Figure 2.4). Unsalted crackers and spring water were provided for cleansing the palate between samples. Panellists were asked to taste the presented sample following the codes written on their screens and answer the questions. The panellists were asked to rate samples for odour, mouth sensation, taste, flavour and aftereffects on a 100-unstructured line scale with anchors from "not" to "very" for the majority of the attributes, except for ripeness were the anchors were from "not" to "overripe". Comments were also collected for each sample.

Figure 2.4. Sensory analysis of the nine genotypes of the strawberry population (RGxH); a) samples presented to the panellists; b) rating session; c) vocabulary development session. Photographs were taken on 10-14.09.2015.

2.6.3 Statistical analysis (3rd year experiment)

The quantitative data (physicochemical traits, non-volatile and volatile compounds) were analysed by both one- and two-way analysis of variance (ANOVA) and Principal Component Analysis (PCA) using XLSTAT Version

2012.1.01 (Addinsoft, Paris, France). For those compounds exhibiting significant difference in the one-way ANOVA, Tukey's test was applied to determine which sample means differed significantly ($P<0.05$). SENPAQ version 3.2 (Qi Statistics, Reading, UK) was used to carry out ANOVA and PCA of sensory panel data. The means for the sensory data were taken over assessors and correlated with the means from instrumental data via PCA using XLSTAT.

The means for the sensory data were taken and used in Principal Component Analysis (PCA, Pearson *n*-1; XLStat) to extract principal components (PCs). Sensory relationships were determined by coefficient analysis. Physicochemical data and headspace volatiles were collated as described in section 2.6. These were regressed onto the sensory PCA as supplementary data, and correlation matrices (Pearson *n*-1; XLStat) were generated to determine significant relationships. Sensory variables with statistically significant correlations were identified at levels of $P<0.05$, <0.01 and <0.001.

Chapter 3 : The impact of genotypes, storage and cultivation sites on post-harvest strawberry quality

3.1 Introduction

Strawberry fruits are very popular in the world, due to which a large number of research studies have been conducted to study the quality traits (fresh weight, firmness, total soluble solids (TSS), titratable acidity (TA), phenolic content and colour) in order to understand the changes of these traits among storage and different cultivation site (Camargo et al., 2011; Crespo et al., 2010; Figueroa et al., 2010; Forney et al., 2000; Gharneh et al., 2012; Gonçalves et al., 2007; Kafkas et al., 2007; Määttä-Riihinen et al., 2004; Majidi et al., 2011; Montero et al., 1996; Nishiyama and Kanahama, 2009; Vicente et al., 2005). However, there is a need for more information that addresses how these quantitative traits are being influenced by genotype (G), environment (E) and their interactions.

In this chapter, the changes in the above mentioned post-harvest quality attributes of the Redgauntlet x Hapil population (RGxH) were studied during two successive harvesting periods at two different sites (season 2013 at East Malling Research and season 2014 Reading; for more details see Chapter 2; sections 2.3.1 and 2.3.2). Site condition differed between the two sites as an open field trial was conducted at EMR (2013), while a glasshouse trial was conducted at Reading (2014). Despite the fact that it was not possible to use a totally conserved set of lines in both years, the impact of genotype and environment, including storage

and two cultivation sites, and their interactions on nutritional and quality traits, were assessed.

3.2 Materials and methods

The materials and methods used for the experiments in this chapter are described in detail in Chapter 2.

3.3 Results and discussion

3.3.1 Post-harvest quality traits analysed over two seasons

From two experiments over two sequential years (2013-2014), seven post-harvest traits of the strawberry mapping population derived from the cross of Redgauntlet x Hapil were phenotyped. Traits analysed included fresh weight (FW), colour, firmness, total soluble solids (TSS), titratable acidity (TA), TSS-to-titratable acid ratio (TSS/TA ratio) and phenolic content at different post-harvest days (day 1 and 7 for year 1 and day 1, day 4 and 7 for year 2). The above-mentioned traits were investigated with the aim of discovering the impact of genotype, storage and cultivation site on strawberry fruit quality.

3.3.2 Diversity between the parental lines for quality traits over two sites.

The parents of the population "RG and Hapil" were previously chosen to generate the mapping population based on their trait divergence (for more details see section 2.3.1). Over two seasons, the female parent "RG" was superior (had higher values) to the male parent "Hapil" in some important characteristics linked to fruit quality including; ellagic acid content, pelargonidin content, and cyanidin content

(Table 3.1; fold change > 1). It was also superior in other characteristics including; TSS/TA ratio (season 2013), which is normally associated with best flavour as a high ratio is known precursor of good strawberry taste, TSS and TA (season 2014), L* value "brightness-darkness spectrum" and a* value "green-red spectrum" (season 2014) (Zorrilla-Fontanesi et al., 2011) (Table 3.1; fold change > 1). The male parent "Hapil" exhibited superior fruit quality traits such as; TSS and TA (season 2013), TSS/TA ratio (season 2014), L* and a* values (season 2013), b* value "blue–yellow spectrum" (season 2013 and 2014) (Zorrilla-Fontanesi et al., 2011), FW and firmness (season 2014) (Table 3.1; fold change < 1). There seemed to be a site effect including growing environment conditions between the parental lines, thus more focus on the effect of the cultivation sites on quality traits was evaluated below in this chapter (section 3.3.3).

The most striking difference between the parental lines was found in polyphenol content, especially with regard to anthocyanins (pelargonidin and cyanidin), which are known as the main colour compounds in the plant (Ho, 1992; Seeram et al., 2006). Anthocyanins were the most variable across the parental lines showing 2.91 fold and 2.46 fold concentration for RG for pelargonidin-7-13 and cyanidin-7-13, respectively (Table 3.1). This is in agreement with the fact that the content of phenolic compounds (including phenolic acid and anthocyanins) in strawberry (specifically) and berries (generally) vary with cultivars (Aaby et al., 2012; Crespo et al., 2010).

Table 3.1. Fold difference between RG and Hapil for quality traits over two seasons.

Traits	Day	Fold change of RG to Hapil	
		Season 2013 (EMR)	Season 2014 (Rdg)
TSS	Day 1	0.94	1.02
	Day 4	-	1.05
	Day 7	0.81	1.36
TA	Day 1	0.90	1.13
	Day 4	-	1.17
	Day 7	0.71	1.16
TSS/TA ratio	Day 1	1.04	0.92
	Day 4	-	0.90
	Day 7	1.15	1.17
L* value	Day 1	0.78	1.07
	Day 4	-	1.11
	Day 7	0.97	0.97
a* value	Day 1	0.80	1.14
	Day 4	-	1.13
	Day 7	0.89	1.08
b* value	Day 1	0.71	0.96
	Day 4	-	0.91
	Day 7	0.60	0.84
FW	Day 1	-	0.88
	Day 4	-	0.91
	Day 7	-	0.85
Firmness	Day 1	-	0.81
	Day 4	-	0.65
	Day 7	-	0.79
Ellagic acid	Day 1	2.56	0.76
	Day 4	-	1.06
	Day 7	2.44	1.65
Pelargonidin	Day 1	1.17	0.63
	Day 4	-	1.24

Traits	Day	Fold change of RG to Hapil	
		Season 2013 (EMR)	Season 2014 (Rdg)
	Day 7	2.91	1.22
Cyanidin	Day 1	1.32	0.76
	Day 4	-	1.48
	Day 7	2.46	1.66

For 2013, only two post-harvest time points were possible (day 1 and 7) because of the limited number of harvested fruits. FW and firmness were not analysed in 2013. n = 4 for sugar, acid and colour measurements, n = 2 for polyphenols. For 2014, n = 6 for FW, firmness and colour measurements, n = 4 for sugar and acids, n=2 for polyphenols.

3.3.3 Impact of cultivation site on post-harvest quality of strawberry.

Previously, the influence of cultivation site on the nutritional and quality traits in strawberry was assessed using different cultivars (Anttonen et al., 2006; Carbone et al., 2009; Cardeñosa et al., 2016; Cocco et al., 2015; Crespo et al., 2010; Häkkinen, 2000; Häkkinen and Törrönen, 2000; Hernanz et al., 2007; Josuttis et al., 2012; Krüger et al., 2012; Wang and Millner, 2009; Zheng et al., 2009). Different environmental conditions such as soil composition (Josuttis et al., 2012), temperature, day length, and light quality and quantity are changing with different cultivation sites (Jaakola and Hohtola, 2010). The effect of cultivation sites on post-harvest quality traits was investigated in this experiment using two different sites East Malling Research (direct planting into open field) and University of Reading (pot grown in glasshouses). Twenty overlapping lines, those grown at both sites, of the RG x H population including the parents were assessed to evaluate the impact of cultivation site on strawberry quality traits (Figure 3.1; for

more details about the characteristics of the two sites see section 2.3). These overlapping lines represent the phenotypic diversity across the whole population (for more evidence refer to section 3.3.5; Figures 3.4 – 3.11). The analysis of variance (ANOVA) revealed that the post-harvest quality traits were significantly affected by environmental factors ($p < 0.05$), except for the trait of fruit lightness (L* value) that did not have significant variation between sites (Table 1.2).

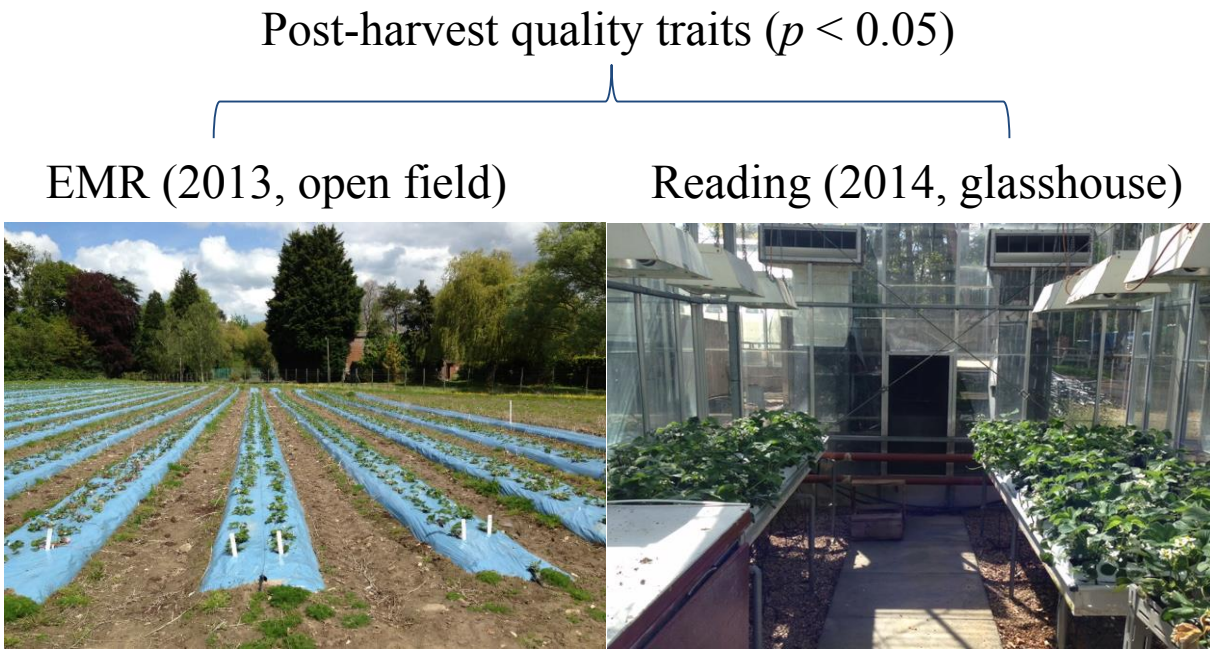


Figure 3.1. Significant differences between the two cultivation sites on post-harvest quality traits (EMR and Reading). ANOVA of TSS, TA and TSS/TA ratio a* value, b* value, ellagic acid, pelargonidin and cyanidin at two cultivation sites (EMR and Reading) of 18 F1 overlapping lines plus the parents.

To compare the EMR and Reading field trials, only the data of day 1 were analysed for the 18 lines grown on both sites, plus the parents. The differences between the two sites for the parental lines were significant at $p<0.05$ for the traits of TA, L* value, a* value, pelargonidin and cyanidin. ANOVA showed that there

were high significant differences among the 20 overlapping lines including the parents between EMR and Reading sites for TSS, TA, colour, and polyphenols, except for the trait of fruit lightness (L* value) ($p<0.001$; Figure 3.1; Table 3.2). The means of F1 individuals for the traits were approximately equal to the mean of the two parental lines in some traits such as TSS, L* value, a* value and pelargonidin (Table 3.2). More details are described below for each trait separately.

Table 3.2. Table of means and range value for quality traits of the overlapping F1 and parent lines grown on two different sites (General ANOVA; $p < 0.05$). Mean and range values for measured traits of the mapping population and parents; Ellagic acid, pelargonidin and cyanidin content (mmol/g FW), TSS (ºBRIX), TA (%), and FW (g).

Traits	sites	Parents			F1 lines grown on both sites			ANOVA		
		RG	Hapil	Mean	Min	Max	Mean	Genotype (G)	Site (E)	G x E
TSS	EMR	7.93	8.48	8.20	6.95	10.93	8.94	<.001***	<.001***	<.001***
	Rdg	9.28	9.13	9.21	5.08	11.28	8.18			
TA	EMR	0.82	0.91	0.86	0.56	0.91	0.73	<.001***	<.001***	<.001***
	Rdg	1.05	1.05	1.05	0.48	1.08	0.78			
TSS/TA%	EMR	9.81	9.46	9.64	9.46	18.74	14.1	<.001***	<.001***	<.001***
	Rdg	8.98	9.96	9.47	8.02	17.42	12.72			
L* value	EMR	29.22	37.59	33.41	28.97	41.61	35.29	<.001***	NS	0.002*
	Rdg	36.36	35.49	35.93	30.74	40.71	35.73			
a* value	EMR	17.98	22.43	20.21	15.16	29.05	22.11	<.001***	<.001***	<.001***
	Rdg	29.9	24.84	27.37	18.01	30.48	24.25			
b* value	EMR	13.68	19.42	16.54	9.31	10.61	9.96	<.001***	0.008*	NS
	Rdg	19.06	19.03	19.05	10.35	13.41	11.88			
Ellagic acid	EMR	4.27	1.67	2.97	1.06	12.88	6.97	<.001***	<.001***	<.001***
	Rdg	2.31	2.88	2.59	1.4	6.59	3.99			
Pelargonidin	EMR	7.61	6.48	7.04	0.66	12.55	6.6	<.001***	<.001***	<.001***
	Rdg	2.96	4.65	3.8	0.23	7.76	3.99			

Traits	sites	Parents			F1 lines grown on both sites			ANOVA		
		RG	Hapil	Mean	Min	Max	Mean	Genotype (G)	Site (E)	G x E
Cyanidin	EMR	0.78	0.59	0.68	0.26	1.16	0.71	<.001***	<.001***	<.001***
	Rdg	0.39	0.49	0.44	0.24	1.69	0.96			

***** Significant, ******* high significant, **NS** = not significant.

3.3.3.1 Phenolic compounds

Strawberry cultivated in EMR in 2013 had significantly higher phenolic compounds compared to those cultivated in Reading in 2014 for both parental lines ($p < 0.001$; Table 3.2). This difference was due to the higher concentrations of ellagic acid, pelargonidin and cyanidin in the strawberries grown in EMR compared to those grown in Reading. Strawberries contain both ellagic acid and its glucoside. Ellagic acid content in strawberry fruits in EMR ranged from 1.06 to 12.88 mmol/g FW, while in Reading ranged from 1.4 to 6.59 mmol/g FW, suggesting that environmental factors have an influence on the phenolic content. The mean ellagic acid content among the overlapping lines was 6.97 and 3.99 mmol/g FW for EMR and Reading, respectively. Among the parental lines, RG had higher amounts of ellagic acid than fruits of the other parent "Hapil".

Values of ellagic acid content, ranging from 1.06-12.88 mmol/g FW (0.3-3.7 mg/g FW), while in Reading they ranged from 1.4 to 6.59 mmol/g FW (0.4-1.9 mg/g FW), were higher than levels found previously in the literature (0.002 to 0.465 mg/g FW) (Häkkinen et al., 1999, 2000; Häkkinen and Törrönen, 2000; Kosar et al., 2004; Wang, 2007; Williner et al., 2003). Such variability might be attributed to cultivar diversity between our population and previously published cultivars (Aaby et al., 2012).

Phenolic acids are known to act as antioxidants and herbivory defence molecules in plants exposed to any kind of stress (Mithöfer and Boland, 2012; Skłodowska

et al., 2011; Treutter, 2006). This is therefore more likely to explain the higher content of phenolic acids in strawberries grown at EMR in an open field system, comparing to those grown at Reading in a glasshouse system, which could suggest that they exposed to more environmental stress. Regional differences have been reported for the total content of phenolic compounds in strawberries cultivated in different places (Cocco et al., 2015; Häkkinen and Törrönen, 2000; Josuttis et al., 2012). Until now, little is known about the effect of cultivation site on flavonoid biosynthesis (Jaakola and Hohtola, 2010; Josuttis et al., 2012; Krüger et al., 2012), however it is known that high temperature and light intensity stimulate the synthesis of the antioxidants compounds in growing fruits (Ariza et al., 2015; Josuttis et al., 2012; Wang and Zheng, 2001).

Anthocyanin content is important for the attractiveness and quality of strawberry. In this experiment, pelargonidin was the main pigment found, corresponding to almost 8-10 times the amount of cyanidin, within each site (Table 3.2). Comparison of the two cultivation sites showed that EMR had approximately double the relative content of anthocyanins compared to the same lines grown in Reading. The mean pelargonidin content among the overlapping lines was 6.6 and 3.99 mmol/g FW for EMR and Reading, respectively. Such variability in the accumulation of phenolic compounds between the two sites and overlapping lines is suggesting that genotype (G), environment (E) and their interaction (G x E) are important and can affect the relative content of phenolic compounds in strawberry

of the population, with a likely effect of cultivation conditions. Pelargonidin content in strawberry fruits in EMR ranged between 0.66 and 12.55 mmol/g FW (0.19-3.6 mg/g FW), while in Reading ranged between 0.23 and 7.76 mmol/g FW (0.06-2.28 mg/g FW). In general, these anthocyanin contents are higher than those reported by Wang and Zheng (2001), in a study carried out in fruit juice of Earliglow and Kent strawberry (*Fragaria* × *ananassa* Duch.) cultivars.

Crespo et al. (2010) found that the relative distribution of anthocyanin content in four cultivars studied was consistent across the two production sites in Switzerland, suggesting that anthocyanin profile was mainly genetically inherited. However, a significant higher amount of phenolics in strawberries grown in plasticulture was reported comparing to those grown in matted row culture (Wang et al., 2002). It was also reported that anthocyanin content was higher on strawberry fruits grown on plastic mulches comparing to those grown on straw mulches as the plastic mulch may preserve a higher temperature which explains the higher content of phenolic compounds observed in these fruits (Anttonen et al., 2006; Moor et al., 2005; Wang and Zheng, 2001). Cardeñosa et al. (2016) showed a higher phenolic composition, mainly flavonols, in blueberries grown under open field system compared to those grown under plastic tunnel system. They explained the differences between the two system as that blueberries grown in an open field system were exposed to more stress (abiotic or biotic factors)

which in turn induce the synthesis and accumulation of the secondary compounds (Cardeñosa et al., 2016).

The quality and duration of light radiation is important for plant development (Jaakola and Hohtola, 2010). Specifically, the ratio of red (R) to far-red (FR) light is responsible for regulating important aspects of plant development including stem extension, specific leaf area, seed germination, and secondary metabolites (Jaakola and Hohtola, 2010). Despite the weather differences between the sites, it was also reported that shading slightly decreased anthocyanin content in strawberry (Anttonen et al., 2006; Dannehl and Josuttis, 2014; Watson et al., 2002), which occurred with strawberry plants grown at Reading where the glasshouse was exposed to the shading by tall trees beside the field which may also explain the lower content of phenolic compounds at Reading. Other factors will also exert their effect on polyphenol accumulation. The glasshouse system was found to reduce the solar radiation by 30 % or more compared with outdoor field (Cockshull et al., 2015). To validate the previous statement, measurements of photosynthetically active radiation (PAR) were taken using (SKP 200 meter, Skye Instruments Ltd, Powys, UK) at Reading between an open field area and the glasshouse and showed a reduction by 25.4 % of the PAR inside the glasshouse compared to the open field. Thus, the explanation of reduction of polyphenols through the shading and the glasshouse system is that as the photosynthesis, which is important to provide the precursor (primary metabolic products) for secondary

metabolites, was reduced by shading and therefore there was less carbon supply for the biosynthesis of polyphenols (Poiroux-Gonord et al., 2010; Treutter, 2010; Watson et al., 2002). This could suggest that strawberry plants cultivated in an open field with a plastic mulch system in EMR (2013) were exposed to more light comparing to those cultivated in a glasshouse with peat-based grow bags system in Reading (2014), thus giving rise to a greater abundance of polyphenol compounds in the fruit tissue.

3.3.3.2 Total soluble solids, Titratable Acidity & TSS/TA ratio

Strawberry flavour is a combination of volatile compounds, sugar and acid content. Fruit quality using human taste panels is often associated with soluble solids (TSS), titratable acidity (TA) and TSS/TA ratio. Sugar content is responsible for the sweetness, while acid content is responsible for the sourness. In the present work, the cultivation site had a significant effect on the content of TSS among the population ($p < 0.05$; Figure 3.2). The TSS content ranges for the two sites were 6.95-10.93 and 5.08-11.28 ºBRIX for EMR and Reading, respectively. The parental lines both showed significantly higher content of TSS at Reading compared to EMR with 9.28 and 9.13 ºBRIX for RG and Hapil, respectively, while the F1 overlapping lines showed divergent trends between the two sites (Figure 3.2). Over two seasons, the site influence was significant ($p < 0.05$), however all overlapping lines showed varying TSS content by site suggesting that the genotyped influence was stronger. The mean of the TSS

content of overlapping lines for EMR site was greater than RG with 8.94 and 8.18, respectively. The highest value at Reading was for RG127 (11.28 ºBRIX), while at EMR was for RG067 (10.93 ºBRIX). The lowest value was observed at Reading for RG125 with 5.8 ºBRIX, while at EMR was for RG100 (6.93 ºBRIX).

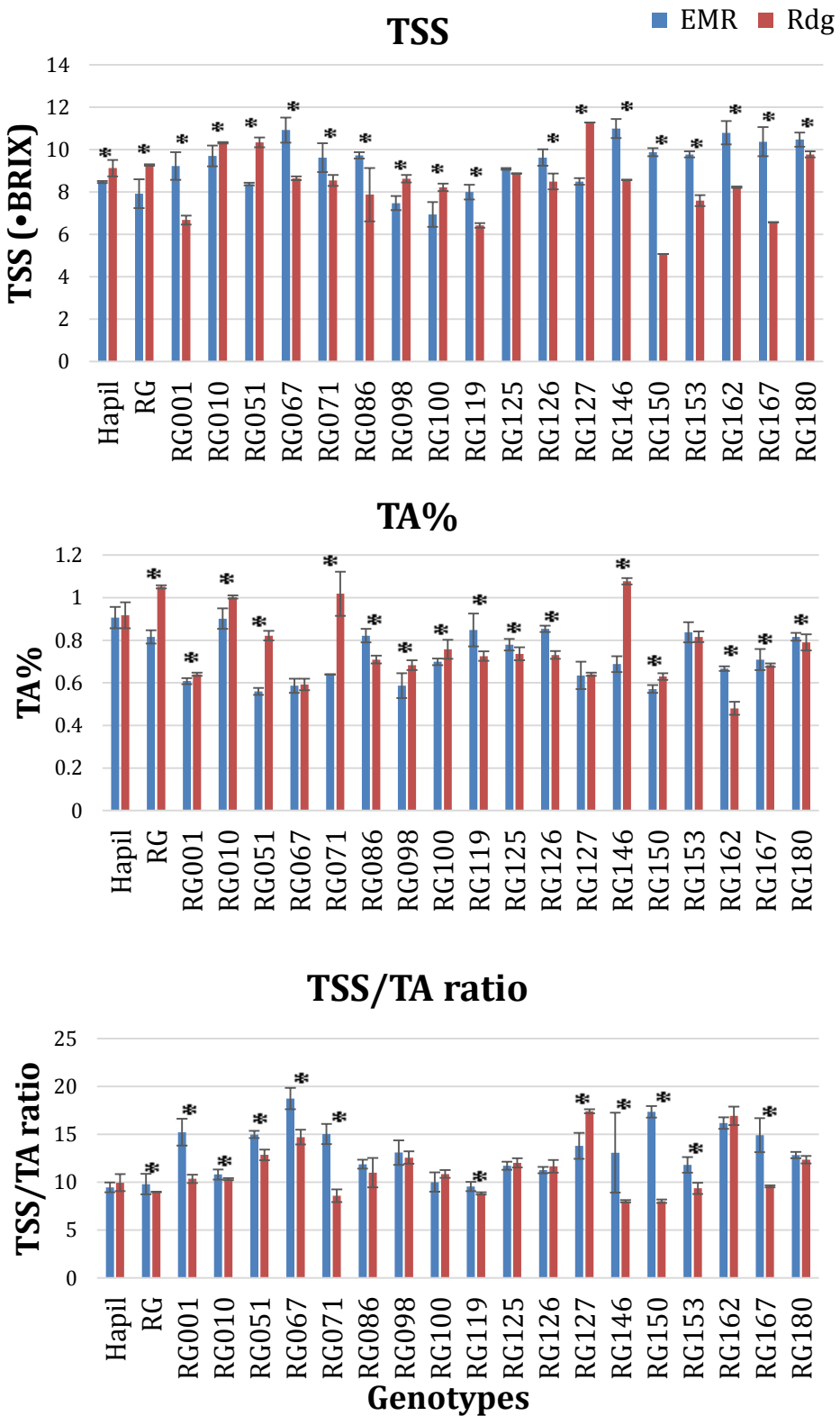


Figure 3.2. Mean of TSS, TA and TSS/TA ratio content at two cultivation sites of the parents and 20 F1 overlapping lines. Error bars are the standard error of means (n = 4). Stars indicate significant differences between sites ($p < 0.05$). LSD $_{TSS}$ = 0.408, LSD $_{TA}$ = 0.02, LSD $_{ratio}$ = 0.706.

Most of the F1 overlapping lines (13 out of 18) grown in EMR had significantly greater TSS content compare to those grown in Reading (Figure 3.2). It was reported that low light exposure might lead to low TSS content by the reduction of photosynthetic rates which lead to less sugar available (Watson et al., 2002). As discussed above (section 3.3.3.1), this may explain the higher TSS content in strawberries grown at EMR compared to those grown at Reading as a result of the shading as well as the glasshouse system that occurred at Reading which reduced the light radiation by 25.4%.

The greatest differences in TSS between cultivation sites were encountered in line RG150 with 1.94 fold greater in EMR (Figure 3.2) compared to when the same line was grown at Reading. Among the 20 overlapping lines, RG125 has a non-significant difference in TSS/TA ratio levels between EMR and Reading (Figure 3.2), which could suggest that this particular line was not affected by the differences between these two growing environments. A previous study on strawberry showed that the production site had a significant effect on the content of monosaccharides among different cultivars (Crespo et al., 2010). Most probably, this resulted from the effect of pre-harvest conditions, including cultivation site, on the respiratory metabolism in which sugars are the main substrate (Crespo et al., 2010; Zheng et al., 2007).

Among the parental lines, the presented data showed that the cultivation sites significantly influenced the parent “RG” for both TA and TSS/TA ratio (Figure

3.2). Both of the parental lines showed higher content of TA at Reading compared to EMR with 1.05 and 0.91 % for RG and Hapil, respectively, with a significant effect for RG only. Previous studies found significant differences in sugar and acids content in strawberries grown at two different sites (Crespo et al., 2010) as well as in TSS and TA content in strawberry (Krüger et al., 2012) and black currant (Zheng et al., 2009), however the observed effects were also cultivar dependant . Crespo et al. (2010) found significant differences in the organic acid content between two different sites, including citric acid and malic acid. Krüger et al. (2012) found that TSS and TA were influenced by latitude giving northern sites (daily mean temperature decreased about 2 °C from south to north) in general the highest values suggesting a positive temperature influence on TSS and TA content.

Among the overlapping lines, most of lines grown in Reading had greater TA content compared to those grown in EMR (Figure 3.2). Additionally, across the whole populations grown each year, fruits grown in Reading showed greater mean of TA content compare to those grown in EMR which may due to the higher fertilizer and irrigation supply in Reading (Table 3.2) (Anttonen et al., 2006; Cocco et al., 2015). The mean TA content among the overlapping lines were 0.73 and 0.78 % for EMR and Reading, respectively, while the mean TA content among the parental lines were 0.82 and 1.05 % for EMR and Reading, respectively (Figure 3.2; Table 3.2). Conversely, most of the overlapping lines

grown in EMR had a higher TSS/TA ratio than when they were grown at Reading, which is mainly due to the lower TA content they have. Shading regime was found to cause a considerable reduction in TSS/TA ratio (Watson et al., 2002), which may be the reason for the increase in the TSS/TA ratio at EMR compared to Reading .

The recommended minimum value of the TSS in strawberries used in commercial practice is 7 ºBRIX, while the maximum value of TA is 8 %, resulting in a value of 8.75 % for TSS/TA ratio (Ayala-Zavala et al., 2004; Camargo et al., 2011). At EMR, all overlapping lines have shown a good balance of sweet and acid as they are all above 8.75 %, however the best balance was attributed to RG067 (18.74 %). While, when grown at Reading, three lines showed bad balance: RG71, RG146, and RG150 scoring values of 8.59, 8.02 and 8.02 %, respectively, suggesting that they were quite acidic. Although ANOVA showed a significant difference between the two sites for TSS, TA, and TSS/TA ratio (Table 3.2), some lines showed non-significant differences (Figure 3.2), which could suggest that those specific lines were not influenced by the environment for these traits.

3.3.3.3 Colour measurements

Changes in colour parameters (L*, a* and b*), where L* value is lightness, a* value is redness-greenness, and b* value is blueness-yellowness, between different sites were also monitored at the point of harvest maturity. In general, significant differences were found in skin colour parameters between the two different sites among the overlapping lines ($p<0.05$) except for fruit brightness (L* value) which could suggest that this parameter is more unlikely to be under the influence of environment, although a significant G x E interaction was detected for L* value (Table 3.2). Overlapping lines showed varying colour parameters (a* and b * values) by site (Figure 3.3). The highest a* and b* values at Reading were found for RG180 (30.48) and RG119 (21.75), respectively, while at EMR were found for RG153 (29.05) and RG153 (22.54), respectively. The lowest a* and b* values were observed at Reading for RG098 (18.01) and RG098 (10.35), respectively, while at EMR was for RG051 (15.16) and RG098 (0.31). Such divergence among the overlapping lines was expected due to the divergence of these parameters between the parents that were used to generate the mapping population.

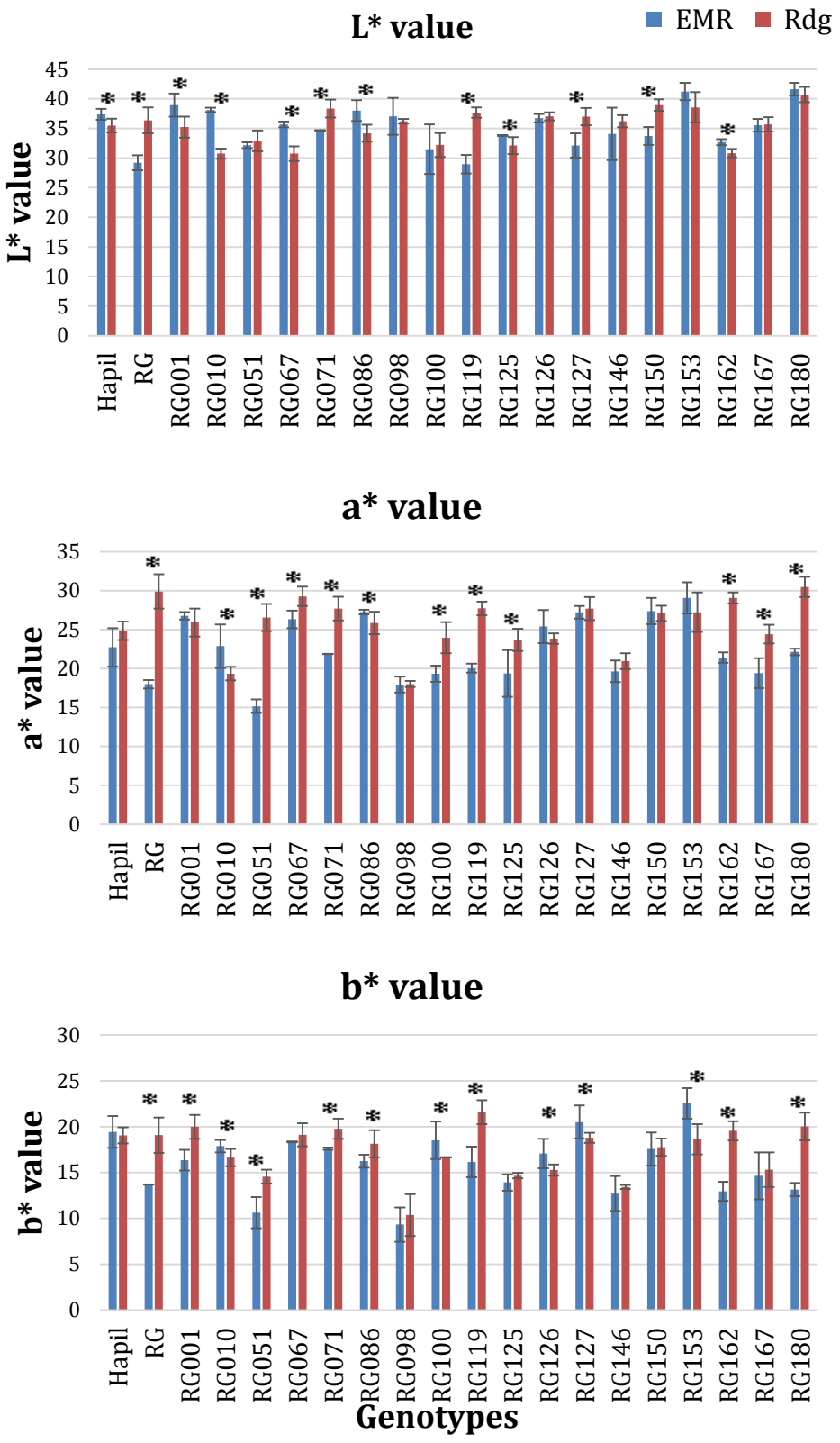


Figure 3.3. Means of colour measurements at two cultivation sites of the parents and 20 F1 overlapping lines. Error bars are the standard error of means ($n_{2013} = 4$; $n_{2014} = 6$). Stars indicate significant differences between sites ($p < 0.05$). LSD $_{L^*} = 1.148$, LSD $_{a^*} = 1.026$, LSD $_{b^*} = 1.058$.

Among the parental lines, RG showed a significant higher values for all parameters (L*, a* and b*) at Reading which could suggest that RG fruits of Reading are less dark comparing to RG fruits of EMR, while Hapil showed a significantly higher value for L* value (less dark) in fruits of EMR compared with those grown in Reading (Figure 3.3; $p < 0.05$). Among the F1 overlapping lines, most of them showed higher values of all colour parameters for fruits cultivated in Reading compared to those cultivated in EMR (Figure 3.3). Additionally, fruits grown in Reading showed greater mean values of colour parameters (35.73, 24.25 and 11.28 for L*, a* and b*, respectively) compared to those grown at EMR (Table 3.2). Although the difference between the two sites for L* value in insignificant, this could suggest that fruits grown in EMR were less dark comparing with those grown in Reading. Interestingly, comparison of the two cultivation sites showed that fruit grown at EMR had also higher anthocyanin content than Reading for both parental lines and overlapping lines. This is in agreement with the findings of Ordidge et al. (2012), where the surface colour was a poor indicator of anthocyanin content.

3.3.4 Impact of genotype on post-harvest quality of strawberry

The assessment of trait variability was conducted across the whole population in both years of assessment (Table 3.2 & 3.3). Results obtained by ANOVA showed that genotype is a strong factor influencing all measured quality traits ($p < 0.001$;

Table 3.2). However, the environmental effect, including cultivation sites and storage, were only significant factors for some traits (Table 3.2 & 3.3).

Among the measured traits, the phenolic content was the most influenced by genotype. For example, the data obtained from season 2013 showed that the F1 progeny showed up to 22.54 fold difference across genotypes between the highest and lowest concentration for pelargonidin on day 7 postharvest (Table 3.3.a). While from data of season 2014, ellagic acid on day 1 showed the largest fold difference up to 33.72 between genotypes (Table 3.3.b). This was unsurprising as the parental lines showed the same trend (for more details refer to 3.3.2). In contrast, the least variable trait in season 2013 was lightness (L* value; day 7) with a 1.48 fold difference, whereas in data of season 2014 fruit lightness (L* value; day 4) was the smallest change with a 1.39 fold difference. Showing such findings indicate that the chemical traits (total soluble solids (TSS), titratable acidity (TA), phenolic content) are strongly influenced by the genetic variation which could suggest that these traits appear to have a strong genetic component determining their regulation. Previously, the genetic variation was reported as the main source of variability in chemical composition of strawberry, including polyphenolic content and profile (Aaby et al., 2012; Atkinson et al., 2006; Cardeñosa et al., 2016; Cocco et al., 2015; Häkkinen and Törrönen, 2000; Josuttis et al., 2012; Meyers et al., 2003; Olsson et al., 2004; Wang, 2007), TSS and TA in berry fruits (Crespo et al., 2010; Gharneh et al., 2012). Such genetic variation

could affect the synthesis and accumulation of biochemical components of strawberry as these processes are under the regulation of specific genes.

Table 3.3. Summary of mean and range values for measured traits of the F_1 population and parents. Mean and range values for measured traits of the mapping population and parents. a) Data of season 2013 at ERM and b) Data of season 2014 at reading. Ellagic acid, pelargonidin and cyanidin content (mmol/g FW), TSS (ºBRIX), TA (%), FW (g), and firmness (N).

a) Data from season 2013

Traits	Day	Parents			F1 population				ANOVA		
		RG ± SD	Hapil ± SD	Mean	Min	Max	Mean	Fold difference	Genotype (G)	Day (E)	G × E
TSS	Day 1	7.93 ± 0.10	8.48 ± 1.35	8.20	6.95	12.20	9.58	1.76	<.001***	NS	0.003*
	Day 7	6.58 ± 0.21	8.08 ± 0.47	7.33	6.58	12.78	9.68	1.94			
TA	Day 1	0.82 ± 0.10	0.91 ± 0.06	0.86	0.50	0.91	0.70	1.83	<.001***	<.001***	<.001***
	Day 7	0.73 ± 0.02	1.03 ± 0.08	0.88	0.47	1.08	0.77	2.30			
TSS/TA ratio	Day 1	9.81 ± 0.96	9.46 ± 1.98	9.64	9.46	24.71	17.09	2.61	<.001***	<.001***	<.001***
	Day 7	9.07 ± 0.30	7.86 ± 0.24	8.47	7.86	22.94	15.40	2.92			
L* Value	Day 1	29.22 ± 1.81	37.59 ± 2.53	33.41	27.92	45.53	36.73	1.63	0.006*	<.001***	0.001***
	Day 7	34.67 ± 4.45	35.77 ± 0.66	35.22	28.98	42.75	35.87	1.48			
a* Value	Day 1	17.99 ± 4.26	22.43 ± 2.04	20.21	15.16	32.82	23.99	2.16	0.04*	<.001***	NS
	Day 7	21.14 ± 2.86	23.71 ± 2.84	22.43	14.91	31.44	23.18	2.11			
b* Value	Day 1	13.69 ± 3.44	19.39 ± 0.03	16.54	9.31	25.81	17.56	2.77	0.023*	<.001***	NS
	Day 7	11.61 ± 2.35	19.40 ± 1.70	15.51	7.58	24.40	15.99	3.22			
Ellagic acid	Day 1	4.27 ± 0.99	1.67 ± 0.14	2.97	1.06	12.88	6.97	12.19	<.001***	NS	NS
	Day 7	3.25 ± 0.20	1.33 ± 0.38	2.29	0.75	10.91	5.83	14.58			
Pelargonidin	Day 1	7.61 ± 0.57	6.48 ± 0.15	7.04	0.66	12.55	6.60	19.13	<.001***	NS	NS

Traits	Day	Parents			F1 population				ANOVA		
		RG ± SD	Hapil ± SD	Mean	Min	Max	Mean	Fold difference	Genotype (G)	Day (E)	G × E
Cyanidin	Day 7	9.69 ± 1.11	3.33 ± 0.54	6.51	0.47	10.68	5.58	22.54	<.001***	NS	NS
	Day 1	0.78 ± 0.05	0.59 ± 0.08	0.68	0.26	1.16	0.71	4.38			
	Day 7	0.86 ± 0.01	0.35 ± 0.04	0.61	0.31	1.63	0.97	5.27			

***** Significant, ******* high significant, **NS** = not significant.

b) Data from season 2014

Traits	Day	Parents			F1 population				ANOVA		
		RG	Hapil	Mean	Min	Max	Mean	Fold difference	Genotype (G)	Day (E)	G × E
TSS	Day 1	9.28 ± 0.78	9.05 ± 0.07	9.16	5.00	11.20	8.10	2.24	<.001***	<.001***	<.001***
	Day 4	9.25 ± 0.13	8.80 ± 0.28	9.03	5.65	11.30	8.48	2.00			
	Day 7	9.80 ± 0.77	7.20 ± 0	8.50	4.85	13.30	9.08	2.74			
TA	Day 1	1.05 ± 0.12	0.93 ± 0.01	0.99	0.49	1.16	0.83	2.37	<.001***	<.001***	<.001***
	Day 4	1.09 ± 0.15	0.93 ± 0.07	1.01	0.53	1.22	0.87	2.28			
	Day 7	1.10 ± 0.11	0.95 ± 0.01	1.03	0.51	1.38	0.94	2.69			
TSS/TA ratio	Day 1	8.98 ± 1.76	9.75 ± 0.08	9.37	5.27	17.22	11.25	3.26	<.001***	<.001***	<.001***
	Day 4	8.62 ± 0.09	9.53 ± 1.07	9.07	5.42	18.62	12.02	3.43			
	Day 7	8.90 ± 0.33	7.59 ± 0.12	8.24	6.85	14.02	10.43	2.05			
L* Value	Day 1	37.88 ± 3.11	35.30 ± 4.40	36.59	29.49	42.13	35.81	1.43	<.001***	<.001***	NS
	Day 4	36.05 ± 3.74	32.36 ± 3.44	34.21	29.95	41.52	35.74	1.39			
	Day 7	34.83 ± 4.05	35.75 ± 2.15	35.29	28.36	40.85	34.61	1.44			
a* Value	Day 1	28.38 ± 3.46	24.85 ± 3.01	26.62	17.53	32.65	25.09	1.86	<.001***	<.001***	NS
	Day 4	27.98 ± 2.01	24.66 ± 1.15	26.32	18.40	31.29	24.85	1.70			
	Day 7	24.72 ± 3.26	22.85 ± 3.19	23.79	14.17	28.59	21.38	2.02			
b* Value	Day 1	18.35 ± 1.95	19.03 ± 3.85	18.69	10.35	24.12	17.24	2.33	<.001***	<.001***	NS
	Day 4	16.62 ± 3.31	18.24 ± 4.24	17.43	9.33	20.96	15.15	2.25			
	Day 7	13.25 ± 2.58	15.76 ± 2.47	14.51	8.52	19.71	14.12	2.31			

Traits	Day	Parents			F1 population				ANOVA		
		RG	Hapil	Mean	Min	Max	Mean	Fold difference	Genotype (G)	Day (E)	G × E
FW	Day 1	12.82 ± 3.57	14.57 ± 1.76	13.70	5.67	18.07	11.87	3.18	<.001 ***	0.03*	NS
	Day 4	12.2 ± 3.36	13.43 ± 1.96	12.82	5.17	17.43	11.30	3.37			
	Day 7	11.58 ± 3.22	13.67 ± 1.55	12.63	4.97	15.67	10.32	3.15			
Firmness	Day 1	8.87 ± 0.68	10.98 ± 3.77	9.93	7.28	12.46	9.87	1.71	<.001 ***	<.001***	0.003*
	Day 4	6.19 ± 1.79	9.55 ± 0.95	7.87	3.53	12.29	7.91	3.47			
	Day 7	6.45 ± 2.64	8.14 ± 0.88	7.30	0.93	12.06	6.50	12.93			
Ellagic acid	Day 1	2.31 ± 0.31	3.03 ± 1.49	2.67	1.40	6.59	3.99	4.71	<.001 ***	NS	NS
	Day 4	5.47 ± 0.45	5.16 ± 2.56	5.32	0.85	8.25	4.55	9.68			
	Day 7	6.12 ± 1.36	3.72 ± 1.83	4.92	0.92	7.44	4.18	8.06			
Pelargonidin	Day 1	2.96 ± 0.44	4.68 ± 2.19	3.82	0.23	7.76	3.99	33.72	<.001 ***	<.001***	NS
	Day 4	4.51 ± 0.18	3.65 ± 1.68	4.08	0.53	6.21	3.37	11.70			
	Day 7	5.44 ± 0.23	4.47 ± 2.09	4.96	0.64	6.41	3.52	10.06			
Cyanidin	Day 1	0.39 ± 0.02	0.51 ± 0.25	0.45	0.24	1.69	0.96	7.07	<.001 ***	<.001***	<.001***
	Day 4	0.57 ± 0.01	0.39 ± 0.19	0.48	0.17	1.12	0.64	6.54			
	Day 7	0.93 ± 0.11	0.56 ± 0.28	0.74	0.20	1.28	0.74	6.57			

***** Significant, ******* high significant, **NS** = not significant.

3.3.5 Impact of storage on post-harvest quality of strawberry

The impact of storage (up to 7 days at a commercially relevant temperature of 4 °C) on post-harvest quality traits was assessed across the population (Table 3.4), the scatter plots for changes from day 1 to day 7 in all quality traits are shown in the appendix, sections 3.3. Seven post-harvest days were chosen based on the conclusion of Ayala-Zavala et al. (2004) who found that strawberry (*Fragaria x ananassa* cv. *Chandler*) stored at 5 °C maintained acceptable quality up to 7 days. For the parental lines, significant differences can be seen between day 1 to day 7 in several traits. For RG, significant differences were observed between day 1 and day 7 in b* value and TSS (2013), b* value (2014), and cyanidin (2014), while for Hapil, significant differences were observed in TSS and TSS/TA ratio for season 2014 (Table 3.5). However, among the full population ANOVA test showed significant differences between day 1 to day 7 in mostly all measured quality traits, except for TSS (2013), ellagic acid (2013 & 2014), pelargonidin (2013), and cyanidin (2013) (Table 3.4). More details for each trait separately are described below.

Table 3.4. The trends of the F1 progeny plus the parental lines over two storage points (day 1 and day 7). TSS (ºBRIX), TA (%), TSS/TA ratio (%), polyphenols (mmol/g FW), FW (g), and firmness (N).

Traits	Season	Parents		Percentage of lines increasing or decreasing in value for each specific trait between day 1 → day 7 in F1 population		
		RG	Hapil	Increased	Decreased	ANOVA $_{day}$
TSS	2013	↓*	↓	50%	50%	NS
	2014	↑	↓*	56%	44%	<.001
TA	2013	↓	↑	62%	38%	<.001
	2014	↑	↑	64%	36%	<.001
TSS/TA ratio	2013	↓	↓	29%	71%	<.001
	2014	↓	↓*	41%	59%	<.001
L* Value	2013	↑	↓	38%	62%	0.006
	2014	↓	↑	31%	69%	<.001
a* Value	2013	↑	↑	47%	53%	0.04
	2014	↓	↓	11%	89%	<.001
b* Value	2013	↓	↑	38%	62%	0.023
	2014	↓*	↓	7%	93%	<.001
Ellagic acid	2013	↓	↓	61%	39%	NS
	2014	↑	↑	33%	67%	NS
Pelargonidin	2013	↑	↓	61%	39%	NS
	2014	↑	↓	46%	54%	<.001
Cyanidin	2013	↑	↓	69%	31%	NS
	2014	↑*	↑	38%	62%	<.001
FW	2013	-	-	-	-	-
	2014	↓	↓	0%	100%	<.001
Firmness	2013	-	-	-	-	-
	2014	↓	↓	11%	89%	<.001

***** Indicates significant difference (ANOVA for season 2013 and Tukey Test for season 2014) between day 1 to day 7 ($p<0.05$), **NS** = not significant. Arrows without stars give a possible indication of the direction of change.

3.3.5.1 Total soluble solids TSS

Strawberries with higher soluble solids are generally preferred over lower soluble solids. The total soluble solids content (TSS) during post-harvest storage at 4 ºC showed a decrease in both parental lines except for RG in season 2014 which increased with storage (Figure 3.4 and Table 3.4). In season 2013, both parents exhibited a reduction in the TSS during postharvest storage, however only RG was significant ($p < 0.05$), whereas in season 2014, RG showed an increase but Hapil exhibited a significant decease during storage ($p < 0.05$). Furthermore, divergent results were obtained from the whole population where in season 2013 some F1 individuals (50 % of the progeny) increased and others (50 % of the progeny) decreased during post-harvest storage, however this was non-significant (Table 3.4). The same trend exists in season 2014 where some F1 individuals (56 % of the progeny) increased and others (46 % of the progeny) decreased during post-harvest storage, however this was significant (Table 3.4). Beside the fact that most of the genotypes vary in performance between the two seasons, such variation among the population suggests a genetic variability within the offspring lines due to the divergence of these parameters from the parents that were used to generate the mapping population.

Greater reduction and increase of TSS during postharvest storage were observed among the population in season 2014 comparing to season 2013 (Figure 3.4). For season 2013, the greatest TSS reduction during storage were observed for RG060

and RG036 with a value of -2.75 and -2.5, respectively, whereas the greatest TSS increase in the seven days of postharvest storage was noted for RG113 and RG126 with a value of 1.4 and 3.15, respectively (Figure 3.4). For season 2014, the highest TSS reduction during storage was observed for RG077 and RG010 with a value of -5.65 and -4.1, respectively, whereas the highest TSS increase during storage was noted for RG026 and RG107 with a value of 4 and 6.65, respectively (Figure 3.4). Beside the differences in the TSS content between the genotypes, the minimum TSS result recorded for season 2013 was 6.95 and the maximum was 12.2 for day 1, while for season 2014 the minimum was 5 and the maximum was 11.2 for day 1. The recommended range of the total soluble solids in strawberries used in commercial practice is 7-12 ºBRIX, depending on the genotype (Ayala-Zavala et al., 2004). Accordingly, the parental lines seemed to be commercially acceptable as the TSS content at day 1 (2013) were 7.9 and 8.47 ºBRIX for RG and Hapil, respectively, while at day 1 (2014) were 9.27 and 9.05 ºBRIX for RG and Hapil, respectively. Additionally, 98.6 % of the offspring at day 1 (2013) were within the recommended commercial range (7-12 ºBRIX), while at day 1 (2014) only 69 % of the offspring at day 1 were within the recommended commercial range.

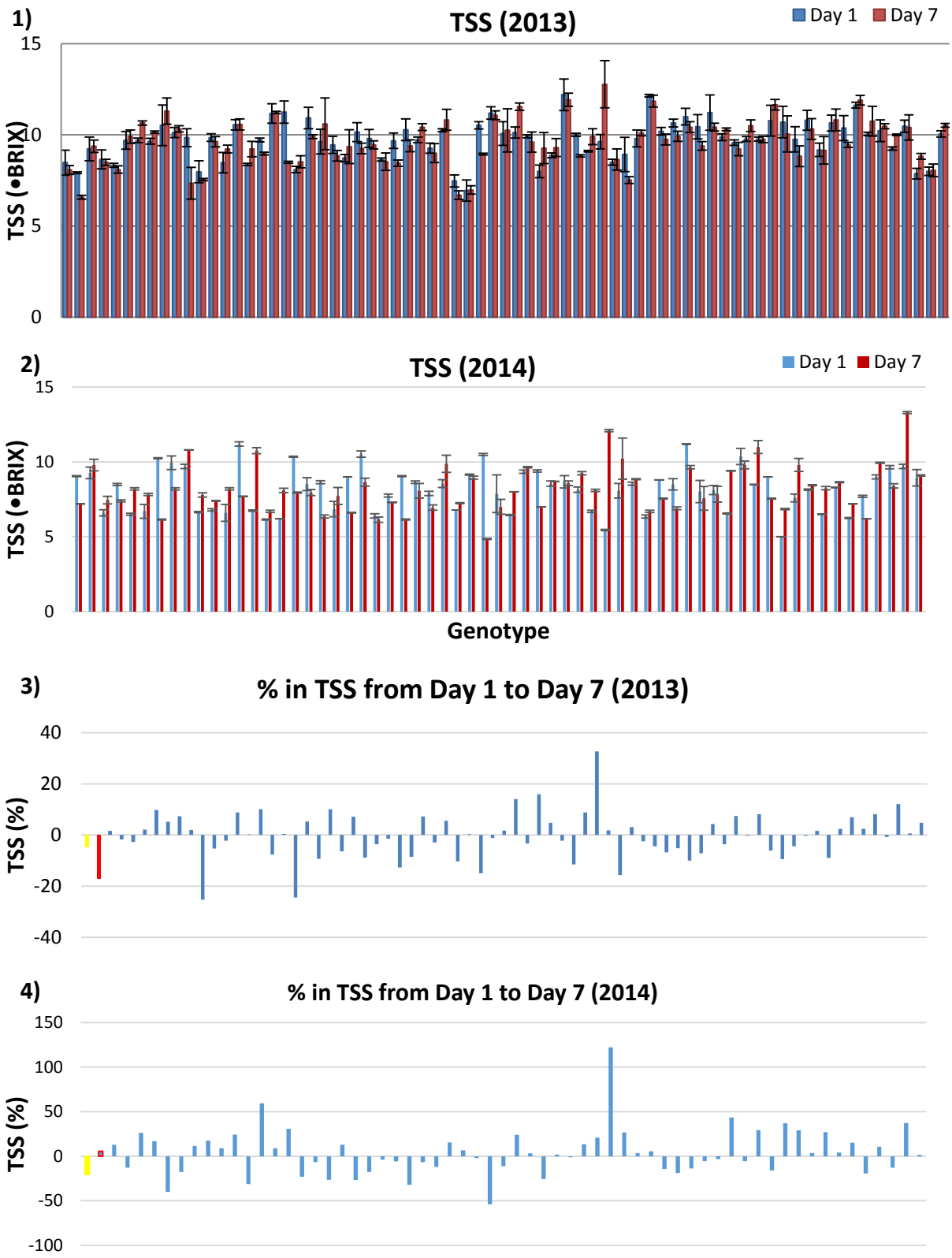


Figure 3.4. Means of TSS (measured as ºBrix) between day 1 and day 7 of the F1 progeny plus the parental lines. (1-2) Differences between day 1 and day 7; (3-4) percentage change from day 1 to day 7. Error bars are the standard error of means (n=4). Red bar is Redgauntlet, yellow bar is Hapil, blue bars are F1 lines.

Among the overlapping lines, the environmental effect between the two sites on the TSS content was evident as most of the lines performed differently during storage for both years ($p < 0.001$; Table 3.2). The TSS content of RG decreased in season 2013 (-1.35 ºBRIX), but increased in season 2014 (0.525 ºBRIX), suggesting the environmental effect. However, the TSS content of Hapil performed similarly during storage as it decreased with -0.4 and -1.85 ºBRIX for 2013 and 2014, respectively (Figure 3.4). Additionally, 11 out of 18 overlapping lines including RG010, RG051, RG086, RG098, RG125, RG126, RG127, RG146, RG162, RG167 and RG180 performed differently for the two years which could suggest that they were under the environmental effect, while RG001, RG067, RG071, RG100, RG119, RG150 and RG153 performed similarly for both years. This was in alignment with the early findings of the environmental effect on TSS content that discussed above in this chapter (refer to section 3.3.3.2).

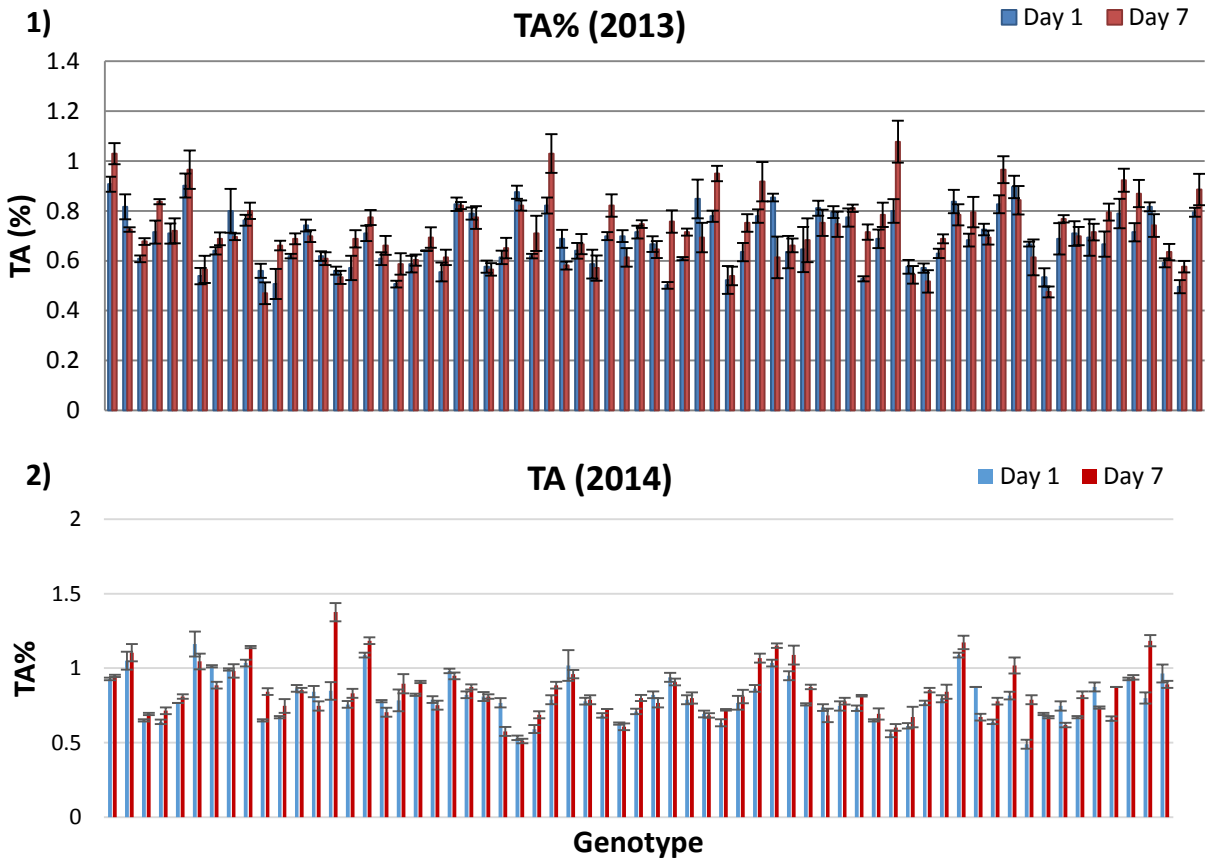
Comparable data reported in the literature showed a decrease in the TSS content of strawberry fruit during storage at 0, 4, 5 and 10 °C, however the reduction was temperature dependant and showed greater magnitude with higher temperature (Ali et al., 2011; Ayala-Zavala et al., 2004; Castro and Goncalves, 2002; Gil et al., 1997; Jouki and Dadashpour, 2012; Mishra and Kar, 2014; Pelayo et al., 2003). The reduction in TSS content during the storage could be attributed to the increasing respiration rate, which is thought to utilize the corresponding reducing sugars (Jouki and Dadashpour, 2012), as well as hydrolysis of sucrose during

storage, as strawberry fruit has very small amount of starch (almost 0.1 %) (Mishra and Kar, 2014; Pelayo et al., 2003). Moreover, minor increase of the TSS content during storage was also previously reported by Cordenunsi et al. (2005) and Jouki and Dadashpour (2012). Such increase was probably due to water loss during storage and hence the increase the concentration of sugar as the strawberries dehydrate (Jouki and Dadashpour, 2012). The explanation of such discrepancy between different studies could be attributed to the conclusion of Watson et al., (2002) who found that TSS, citric acid and volatile compounds varied considerably between cultivars and harvests.

3.3.5.2 Titratable Acidity TA

Titratable acidity was analysed before and after storage at 4 °C for 7 days. TA has been expressed in terms of percentage citric acid since citric acid constitutes the most abundant acid in strawberry (Ali et al., 2011; Mishra and Kar, 2014; Pelayo et al., 2003). The data presented showed that TA content increased during post-harvest storage in the parental lines for both seasons, except for RG in season 2013, however these changes were insignificant which shows a possible indication of the direction of change (Figure 3.5 and Table 3.4). In season 2013, a reduction in TA was shown in RG over seven days of storage periods. This reduction corroborated with previous data from strawberry of different cultivars reported by Pelayo et al. (2003), Cordenunsi et al. (2005) and Mishra and Kar (2014). In contrast to RG, TA content of Hapil increased during storage in 2013;

this was consistent with the view of Camargo et al. (2011) who linked differences in acidity to cultivar variation. However, in season 2014 an increase in TA over storage was shown in both parents, demonstrating that environmental conditions also have a dramatic impact on the final quality of the fruit at harvest to the extent that in both 2013 and 2014 G x E was significant for total acidity (Table 3.2).



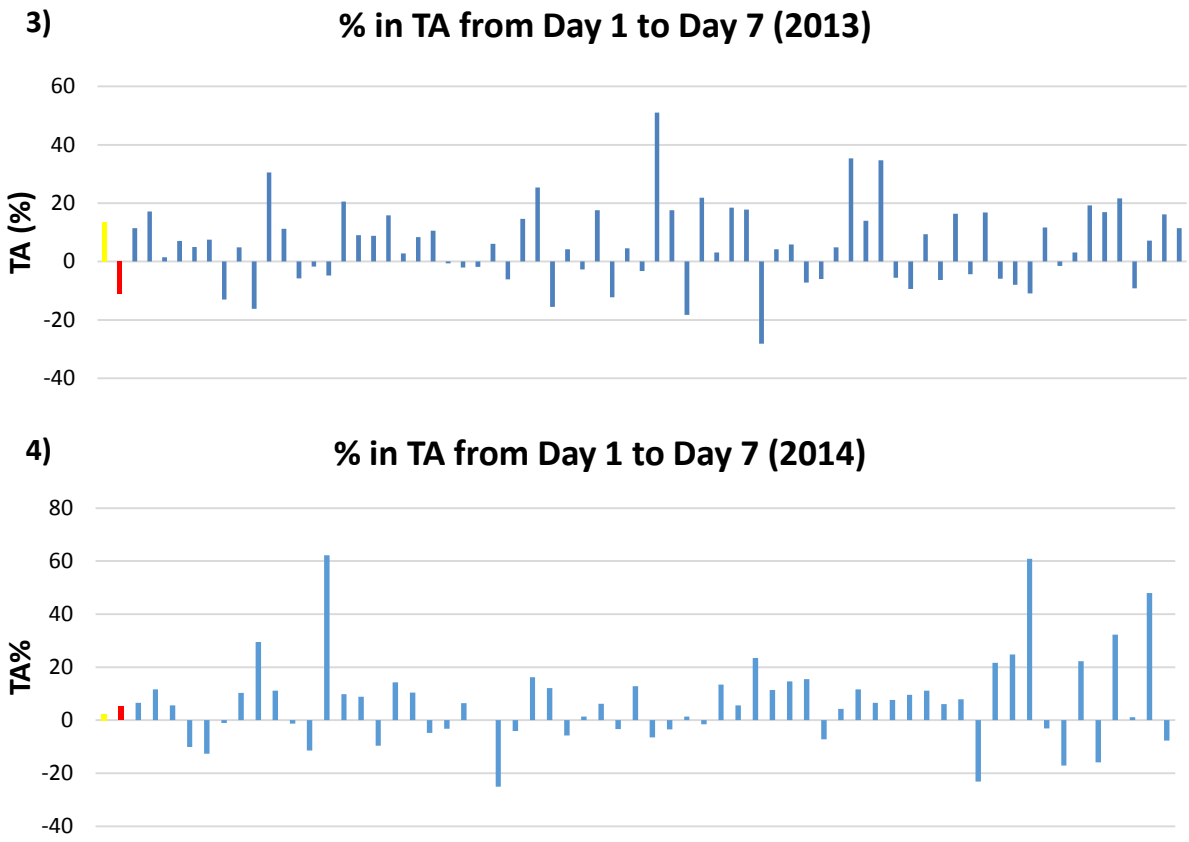


Figure 3.5. Means of TA between day 1 and day 7 of the F1 progeny plus the parental lines. (1-2) Differences between day 1 and day 7; (3-4) percentage change from day 1 to day 7. Error bars are the standard error of means (n=4). Red bar is Redgauntlet, yellow bar is Hapil, blue bars are F1 lines.

Over again, divergent results were obtained from the whole population where in season 2013 some F1 individuals (62 % of the progeny) increased and others (38 % of the progeny) decreased in TA during post-harvest storage (Figure 3.5). The same trend existed in season 2014 where some F1 individuals (64 % of the progeny) increased in TA and others (36 % of the progeny) decreased during post-harvest days (Figure 3.5 and table 3.4). Such variation among the population suggests a genetic variability within the offspring lines due to the divergence of these parameters between the parents that were used to generate the mapping

population. Previously, differences in TA content were reported between two cultivars where they found a decrease in cv. *Camarosa*, while an increase in cv. *Chandler* during storage (Mishra and Kar, 2014).

For season 2013, the greatest TA reduction was observed for RG126 and RG119 with a value of -0.24 and -0.155 %, respectively, whereas the greatest TA increase was noted for RG113 and RG147 with a value of 0.24 and 0.27 % respectively (Figure 3.5). For season 2014, the greatest TA reduction was observed for RG149 and RG064 with a value of -0.203 and -0.192 %, respectively, whereas the greatest TA increase was noted for RG180 and RG026 with a value of 0.384 and 0.528 %, respectively (Figure 3.5). Beside the differences in the TA content between the genotypes, the minimum TA result recorded for season 2013 was 0.49 % and the maximum was 0.90 % for day 1, while for season 2014 the minimum was 0.49 % and the maximum was 1.16 % for day 1.

Among the overlapping lines, the environmental effect between the two sites was evident on the TA content as most of the lines performed differently during storage for both years ($p < 0.001$; Table 3.2). The TA content of RG decreased in season 2013 at EMR (-0.09 %), but increased in season 2014 at Reading (0.05 %), whereas the Hapil performed similarly as the TA content increased with 0.12 % and 0.02 % for 2013 and 2014, respectively, (Figure 3.5). Additionally, 8 out of 18 overlapping lines (RG010, RG051, RG071, RG126, RG150, RG153, and RG162) performed differently during storage for both years which could suggest

that these lines were under the environmental effect, while RG001, RG067, RG086, RG098, RG100, RG119, RG125, RG127, RG146, RG167 and RG180 performed similarly for both years. This was in alignment with the early findings of the environmental effect on TA content which discussed above in this chapter (refer to section 3.3.3.2).

3.3.5.3 TSS/TA ratio

TSS/TA ratio is commonly used as an indicator for consumer satisfaction and strawberry quality (Watson et al., 2002). It is very important parameter as it provides information on the balance of sugars and acids that are linked to strawberry flavour and quality (Crespo et al., 2010; Giné Bordonaba and Terry, 2008; Ana G Pérez et al., 1997; Terry et al., 2005; Zorrilla-Fontanesi et al., 2011). The parent RG retained TSS/TA ratio throughout shelf life for both years which could suggest that RG maintained better taste over the storage as compared with the other parent Hapil (Figure 3.6).

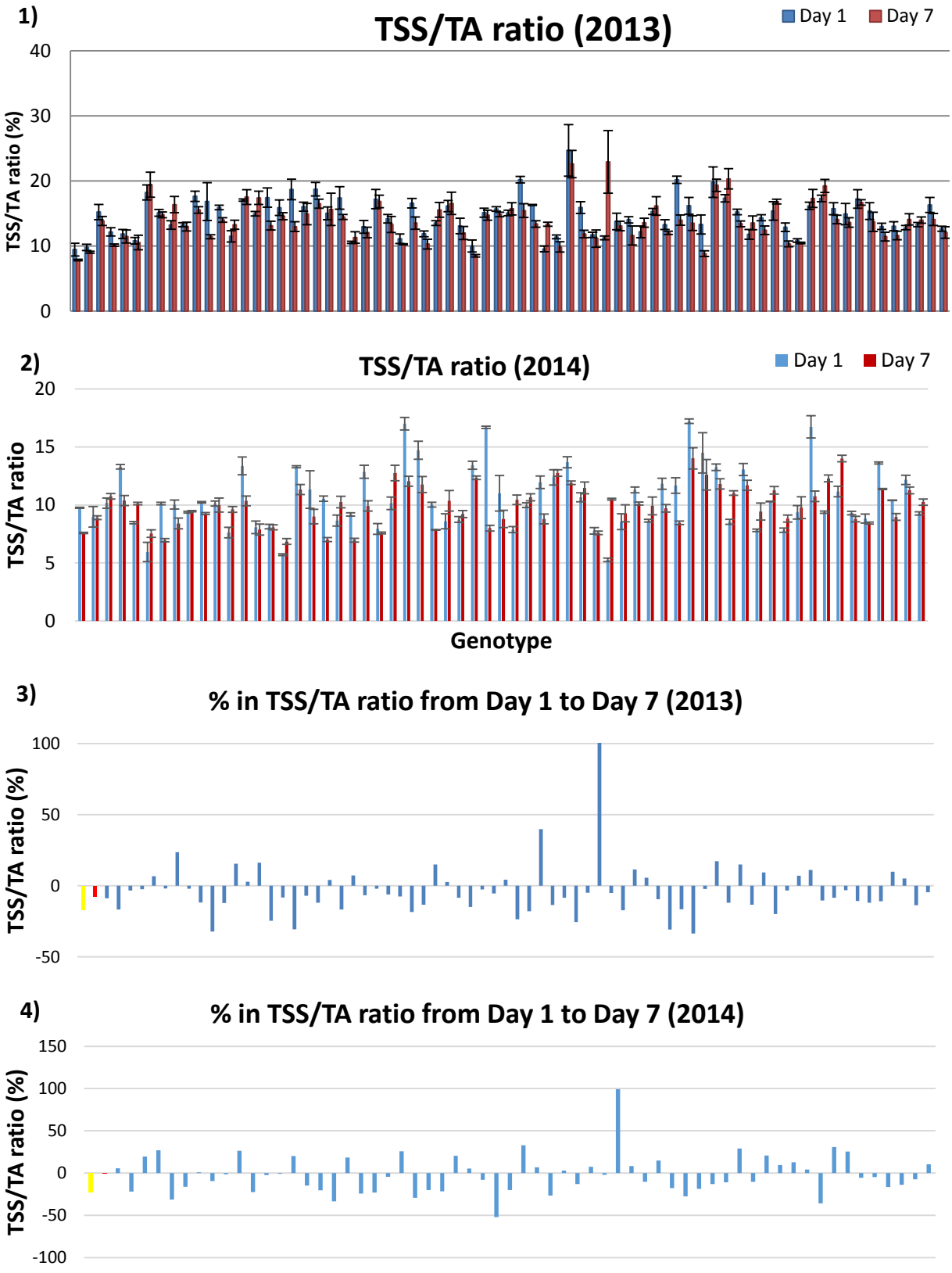


Figure 3.6. Means of TSS/TA ratio between day 1 and day 7 of the F1 progeny plus the parental lines. (1-2) Differences between day 1 and day 7; (3-4) percentage change from day 1 to day 7. Error bars are the standard error of means (n=4). Red bar is Redgauntlet, yellow bar is Hapil, blue bars are F1 lines.

The data presented showed that TSS/TA ratio decreased during post-harvest storage in the parental lines for both seasons, however only Hapil was significant ($p < 0.05$) for season 2014 (Table 3.4). Furthermore, in season 2013 most of the F1 progeny (71 % of the progeny) decreased TSS/TA ratio during shelf life as shown in Figure 3.6 & Table 3.4 ($p < 0.001$). In season 2014 the majority of the F1 progeny (59 %) also decreased TSS/TA ratio over shelf life ($p < 0.001$). TSS/TA ratio is known as the most important taste measurement among fruits (Zorrilla-Fontanesi et al., 2011). This could suggest that lowering TSS/TA ratio values may indicate loss of strawberry taste during storage. This was in agreement with the findings of Voča et al., (2008) who also reported significant differences of post-harvest storage and genotype on TSS/TA ratio in seven different strawberry cultivars.

Among the overlapping lines, environmental effect between the two cultivation sites was evident on the TSS/TA ratio content for 9 out of 18 overlapping lines those performed differently during storage over the two years ($p < 0.001$; Table 3.2). However, both parents performed similarly in the both sites which could suggest that they were unlikely to be under the influence of environment. The TSS/TA ratio content of RG and Hapil decreased in both seasons (2013 and 2014) (Figure 3.6), however the reduction of the TSS/TA ratio throughout shelf life for RG was much less comparing with TSS/TA ratio for Hapil. Additionally, 9 out of 18 overlapping lines including RG001, RG051, RG098, RG100, RG126, RG146,

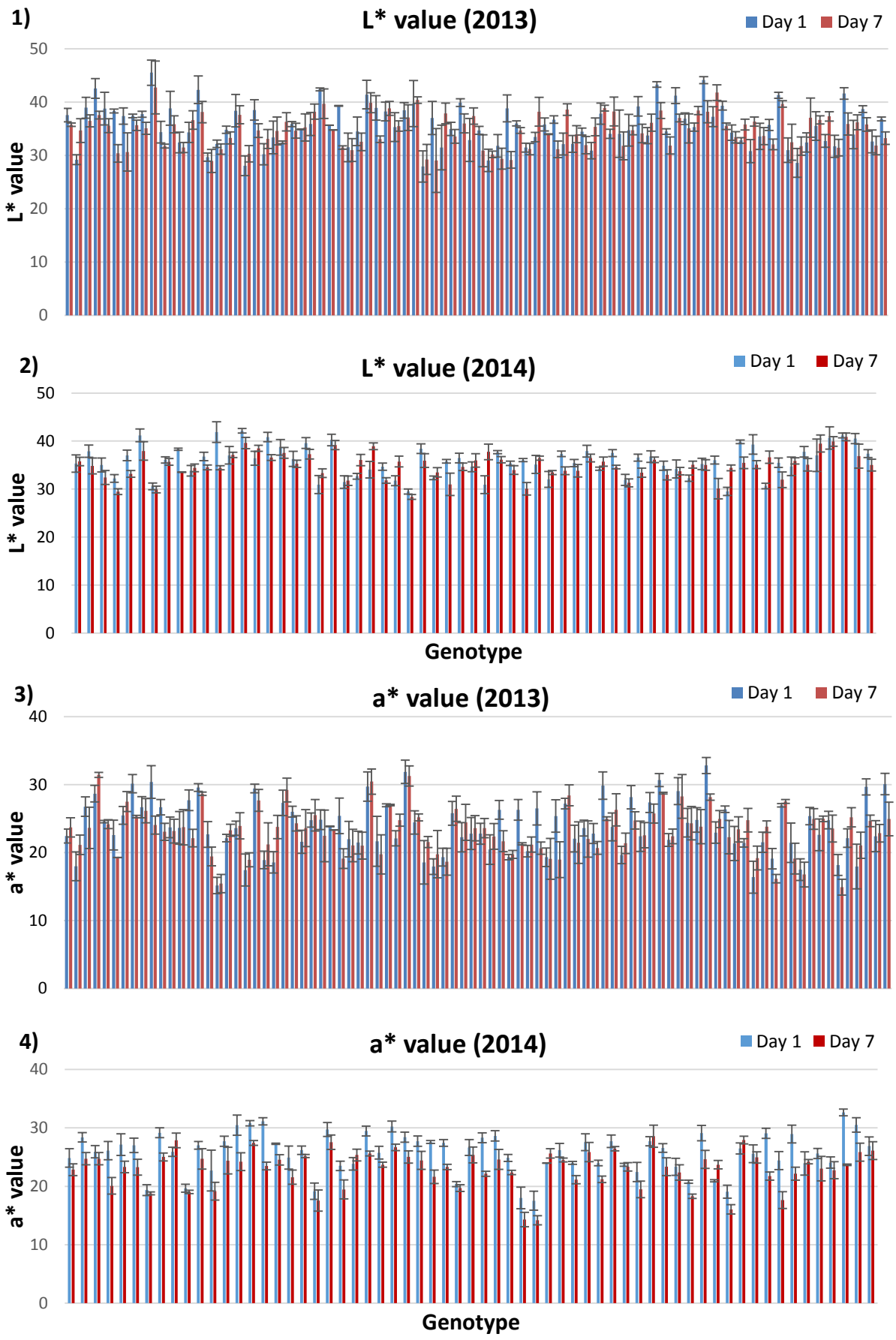
RG162, RG167 and RG180 were under the environmental effect as they all performed differently during storage for the two years, while RG010, RG067, RG071, RG086, RG119, RG125, RG127, RG150 and RG153 performed similarly for both years. This was in alignment with the early findings of the environmental effect on TSS/TA ratio that discussed above in this chapter (refer to section 3.3.3.2).

3.3.5.4 Colour measurements

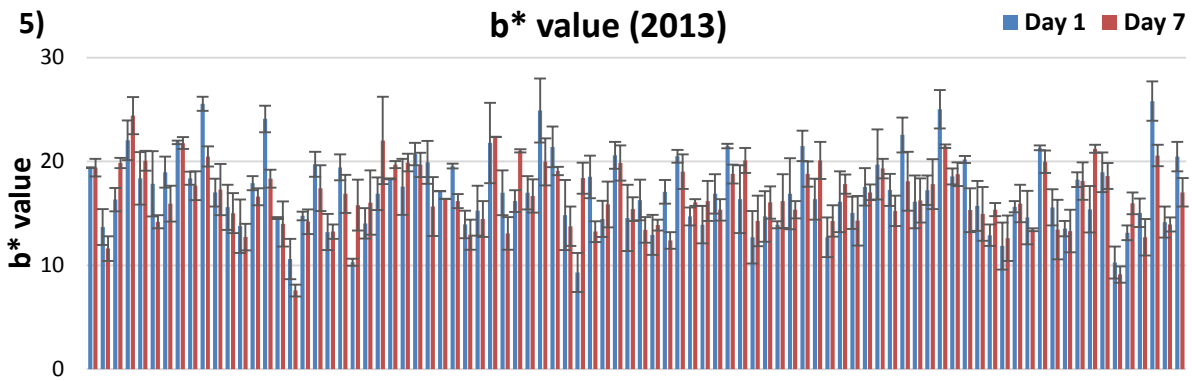
Bright red colour is an important quality parameter that attracts consumers' attention. Therefore, change in colour parameters (L*, a* and b*) during shelf life days were monitored. Significant differences were found in skin colour parameters among genotypes and shelf life storage for both years ($p < 0.05$) (for more details see Table 3.3). Divergent trends were observed from the parental lines for colour parameters (Table 3.4). This divergence might be attributed by the divergence of the parents that were used to produce the mapping population and to capture diversity for these traits in order to be able to conduct QTL analysis (for more details refer to 3.3.2). Changes in colour parameters during shelf life were genotype and environment dependent (Table 3.2 and Table 3.3). The majority of the F1 progeny decreased for all colour parameters during storage in both years (Figure 3.7 and Table 3.4). Such low values of L* (more darkness), a* (less red) and b* (less yellow) at day 7 indicate overall darker fruit colour, in accordance with previous reports (Gil et al., 1997; Kalt et al., 1993; Miszczak et

al., 1995). This is believed to be the result of the accumulation of anthocyanins, which is known as a major pigment in plants, and decrease of chlorophyll synthesis during ripening (Cited by Civello and Martínez, 1997). Anthocyanins production is normally stated to increase at the late stage of ripening "20 to 30 days after petal fall" when the chlorophyll synthesis ceased (Woodward, 1972).

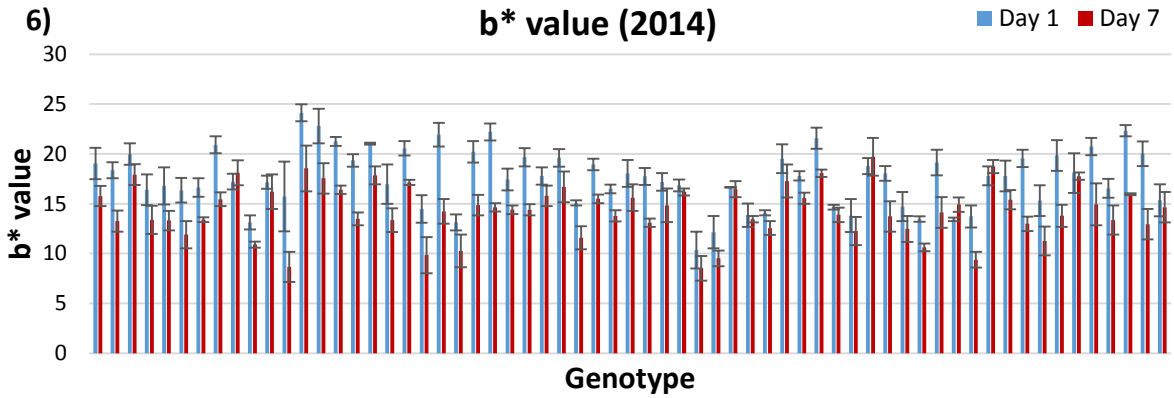
As in the present study, decrease in the L* and a* values of strawberry fruit during storage have previously been reported by others researchers (Jouki and Dadashpour, 2012). Beside the influence of different sites, RG showed a decrease of the L*, a* and b* values (except for L*-2013 and a*-2013) with an increase of anthocyanin content (pelargonidin and cyanidin; refer to 3.3.5.5). Additionally, among the population, most of the progeny lines of season 2013 showed an increase in anthocyanin content (61 % and 64 % of the progeny for pelargonidin and cyanidin, respectively), while most of the progeny showed a reduction in colour parameters (62 %, 53 % and 62 % for L*, a* and b*, respectively). This may explain the role of anthocyanin in the development of red colour in the fruits tested in this thesis; this observation was also reported by Wang et al. (2015).


1)
L* value (2013)
Day 1
Day 7
L* value
50
40
30
20
10
0
2)
L* value (2014)
Day 1
Day 7
L* value
50
40
30
20
10
0
Genotype
3)
a* value (2013)
Day 1
Day 7
a* value
40
30
20
10
0
4)
a* value (2014)
Day 1
Day 7
a* value
40
30
20
10
0
Genotype

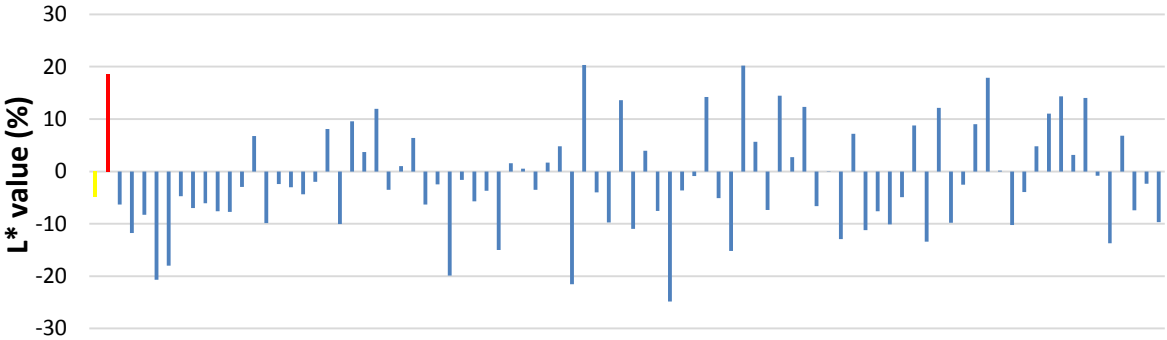
5)


b* value (2013)
Day 1
Day 7
b* value
30
20
10
0

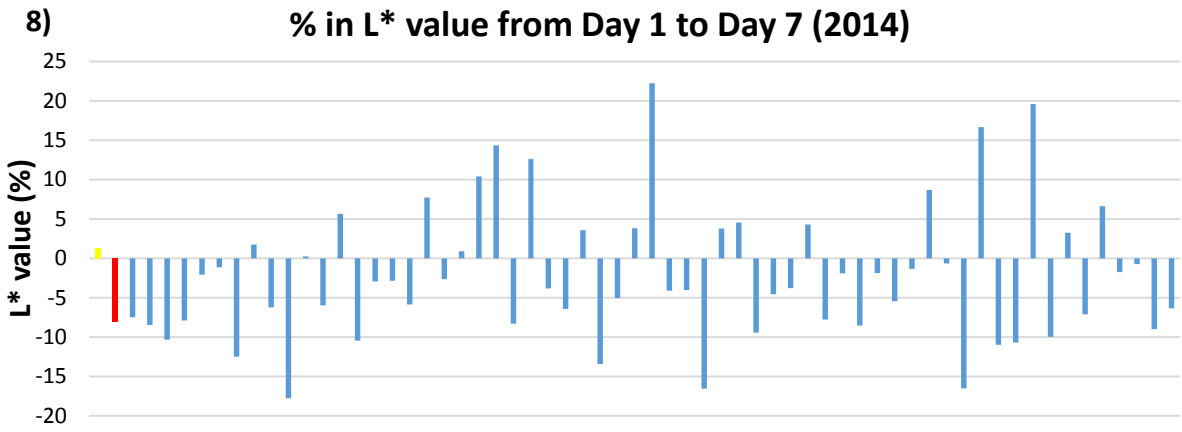
6)


b* value (2014)
Day 1
Day 7
b* value
30
25
20
15
10
5
0
Genotype

7)


% in L* value from Day 1 to Day 7 (2013)
L* value (%)
30
20
10
0
-10
-20
-30

8)


% in L* value from Day 1 to Day 7 (2014)
L* value (%)
25
20
15
10
5
0
-5
-10
-15
-20

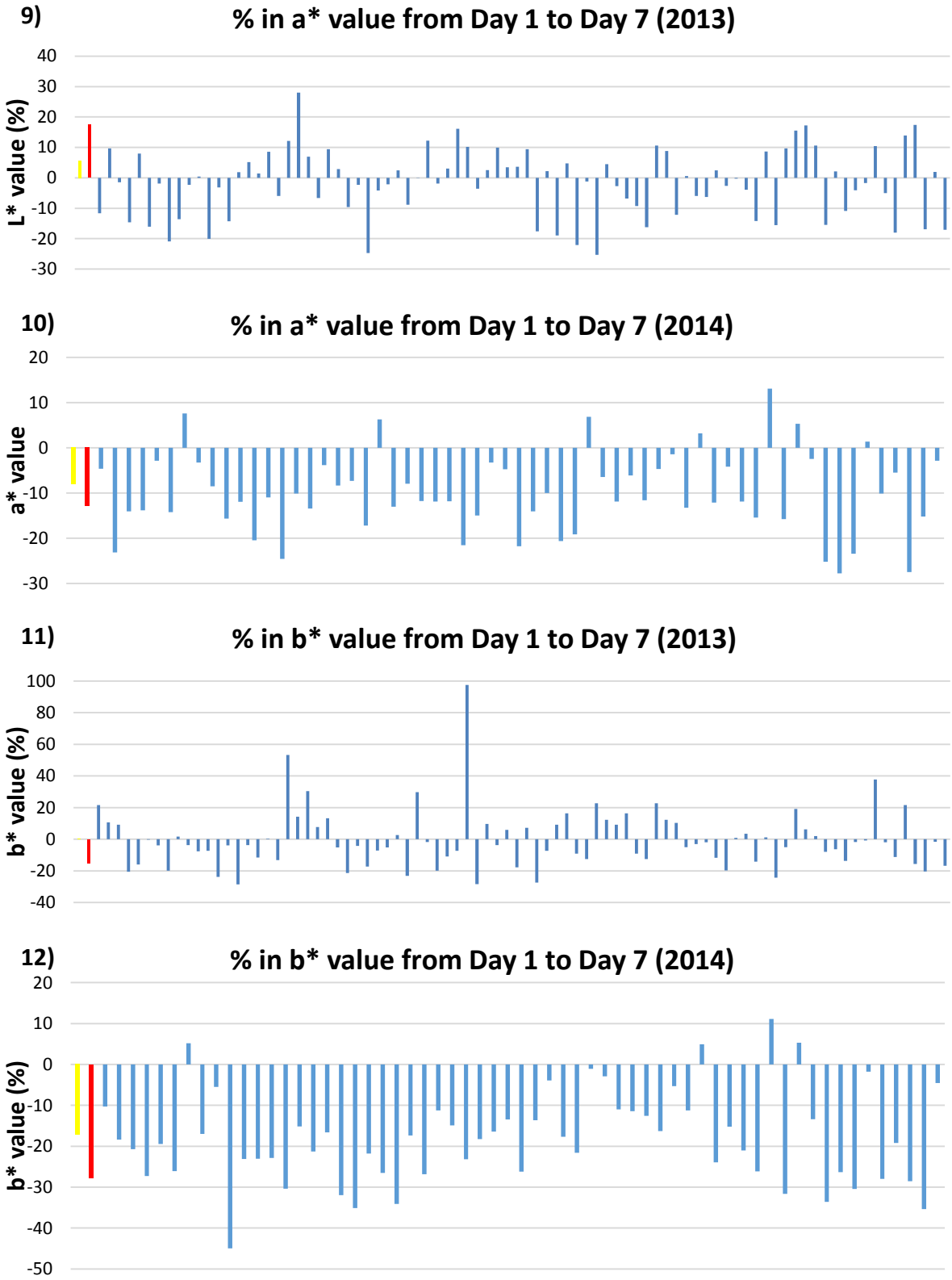


Figure 3.7. Means of L*, a* & b* values between day 1 and day 7 of the F1 progeny plus the parental lines. (1-6) Differences between day 1 and day 7; (7-12) percentage change from day 1 to day 7. Error bars are the standard error of means ($n_{2013} = 4$; $n_{2014} = 6$). Red bar is Redgauntlet, yellow bar is Hapil, blue bars are F1 lines.

For the lightness (L* value), no significant differences were found between the two sites (Table 3.2; for more details, refer to 3.3.3.3). For the red tone (a* value), among the overlapping lines, 11 out of 20 overlapping lines performed differently during storage for both years which could suggest that these lines were under the environmental effect, these lines ($p < 0.001$; Table 3.2). The a* value of RG increased in season 2013 (3.15), but decreased in season 2014 (-3.66), while the a* value of Hapil increased in season 2013 (1.28), but decreased in season 2014 (-2) (Figure 3.7). Additionally, 9 more overlapping lines including RG051, RG071, RG086, RG098, RG100, RG119, RG150, RG162 and RG180, were under the environmental effect as they all performed differently during storage for the two years, while RG001, RG010, RG067, RG125, RG126, RG127, RG146, RG153, and RG167 performed similarly for both years. This was in alignment with the early findings of the environmental effect on colour parameters that discussed above in this chapter (refer to section 3.3.3.3).

For the yellow tone (b* value), among the overlapping lines, 10 out of 20 overlapping lines performed differently during the storage for both years which could also suggest that these lines were under the environmental effect ($p < 0.008$; Table 3.2). The b* value of Hapil did not change in season 2013, but decreased in season 2014 (-3.27), however the RG performed similarly during storage as the b* value increased with -2.08 % and -5.1 % for 2013 and 2014, respectively, which could suggest the dominant role of genotype over environment for this

particular line (Figure 3.7). Additionally, 9 more overlapping lines including RG001, RG067, RG071, RG086, RG098, RG126, RG150, RG162, and RG180, were under the environmental effect as they all performed differently during storage for both years, while RG010, RG051, RG100, RG119, RG125, RG127, RG146, RG150, and RG167 performed similarly for both years. This was in alignment with the early findings of the environmental effect on colour parameters that discussed above in this chapter (refer to section 3.3.3.3). Finally, 5 lines (RG010, RG125, RG146, RG153 and RG167) remained stable during storage over both sites for all colour parameters, which could suggest that these lines may not be influenced by the environmental factors suggesting their ability to tolerate different cultivation conditions in term of colour measurements.

The bright attractive colour of strawberry fruit normally fades and becomes darker with increasing storage period, however, this is mainly depends on storage temperature and light (Kalt et al., 1993). Strawberry colour was reported to become darker and redder when temperature became warmer (Krüger et al., 2012; Miszczak et al., 1995; Wang and Zheng, 2001). Colour development was greater in fruits stored at 20 °C with light comparing to fruits stored at 10 °C in the dark (Miszczak et al., 1995). Furthermore, Krüger et al. (2012) found that strawberry fruits of cvs. *Korona* and *Clery* became darker with increasing temperature and a negative correlation was observed between L* value and temperature. This might be due to the influence of temperature on anthocyanin synthesis which increase

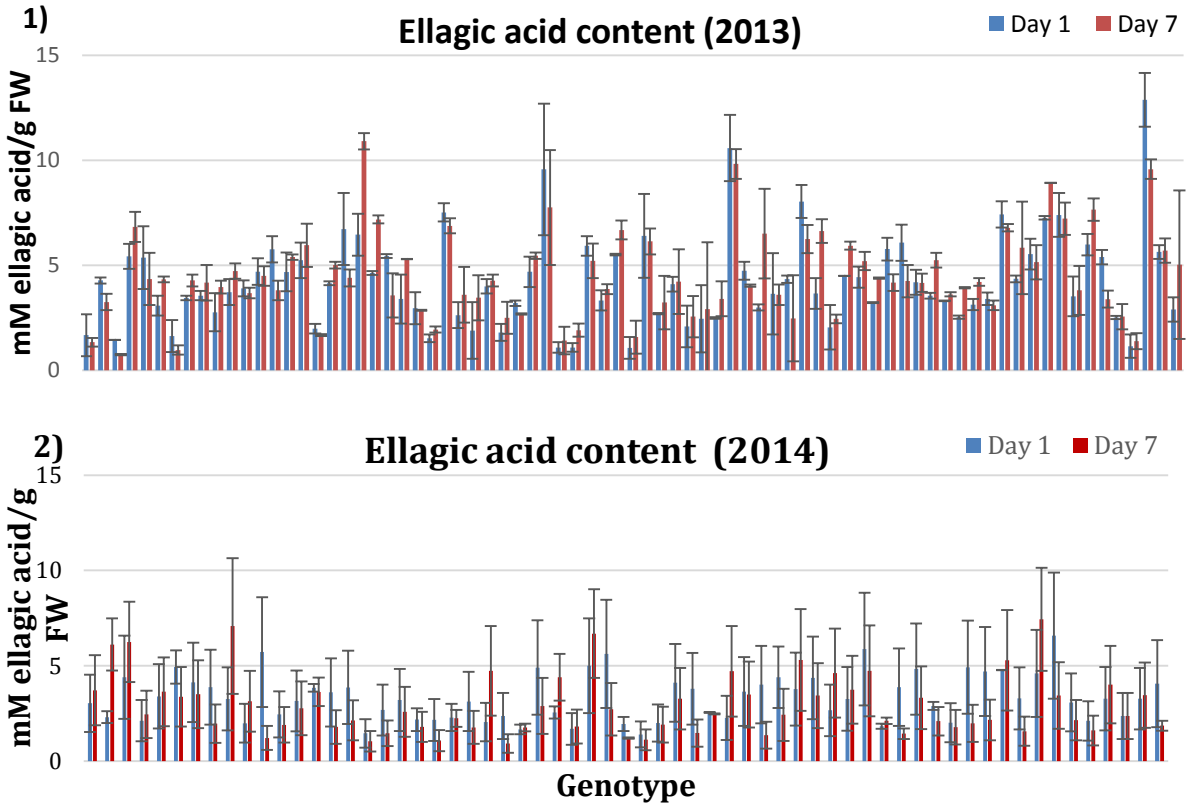
the accumulated content of anthocyanin and in turn the fruit became darker (Cordenunsi et al., 2005). Therefore, an appropriate storage condition during shelf life is important to preserve the red colour and delay colour deterioration.

3.3.5.5 Phenolic compounds

Three major phenolic compounds have been identified over different shelf life days and for both years (2013-2014). These are ellagic acid, which is the major phenolic acid in strawberry, pelargonidin and cyanidin, which are the major anthocyanins in strawberry. No significant differences were found for these compounds during shelf life storage for both years, except for pelargonidin and cyanidin in season 2014 ($p < 0.001$) (Tables 3.3 & 3.4).

The results showed that a reduction of ellagic acid content took place for both parents in season 2013, whilst in season 2014 both parental lines increased in concentration of the same compound, but ANOVA shows this was non-significant (Table 3.4). Such divergent tendencies were also observed among the F1 progeny where in season 2013 61 % increased and 39 % decreased in ellagic acid content, while in season 2014, 33 % increased and 67 % decreased in concentration of the same compound (Figure 3.8 and Table 3.4). Cordenunsi et al. (2005) found a significant decrease in ellagic acid for cv. *Campineiro* upon storage at three temperatures (6, 16, and 25 ° C), while no clear tendency was observed for the other two cultivars of the study (cvs. *Dover* and *Oso Grande)*. However, Ayala-Zavala et al. (2004) reported increasing of phenolic compounds in berries stored

at 10 °C and 5 °C, whereas Häkkinen and Törrönen (2000) reported a 40 % decrease in ellagic acid content in strawberry during 9 months of storage in a freezer. It is well known that strawberry fruits have very low relative amount of free ellagic acid, which is thought to form following hydrolytic release from ellagic acid derivatives including ellagic acid glycosides and ellagitannins, compared to other derivatives and polymerized forms (~5 %) (Häkkinen and Törrönen, 2000), so it could be that such storage condition (4 °C) did not make any significant differences in the free ellagic acid content. These findings compared to the data from this thesis on strawberry may indicate that although storage conditions and longevity have an effect on free ellagic acid content, the concentration is more dependent on cultivar.



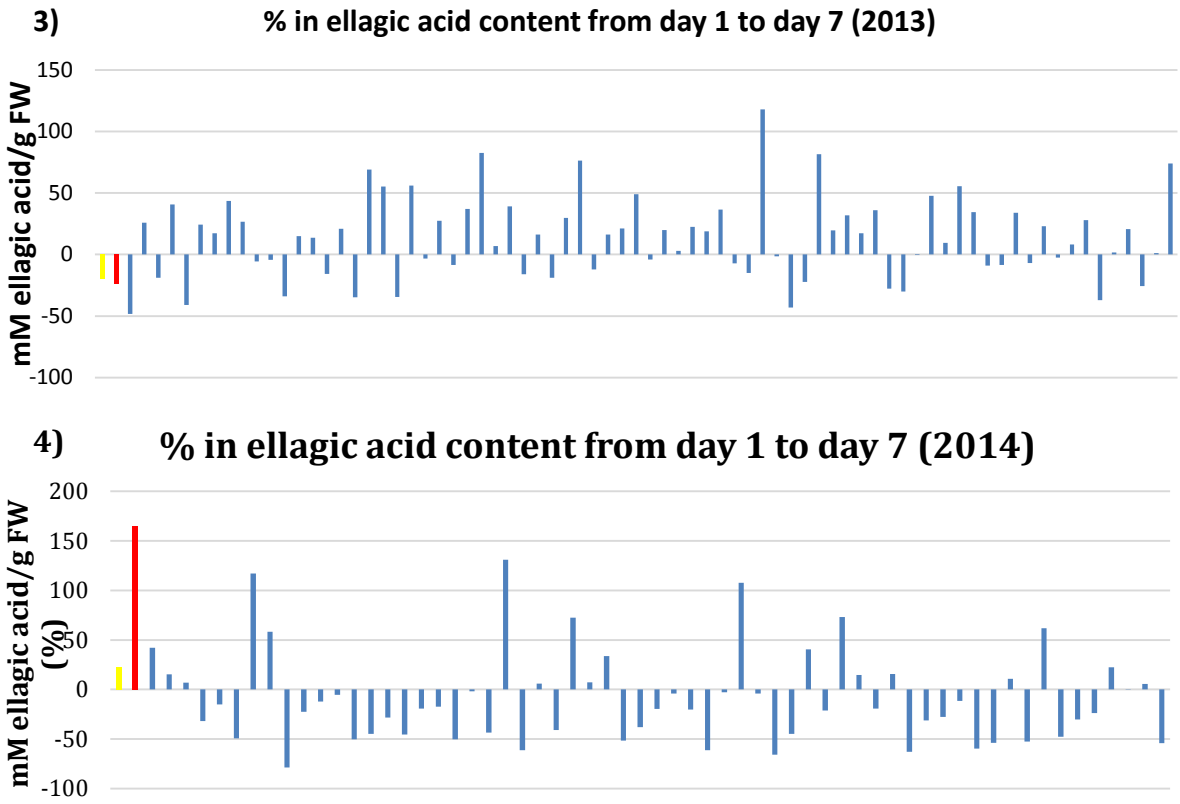


Figure 3.8. Means of ellagic acid between day 1 and day 7 of the F1 progeny plus the parental lines. (1-2) Differences between day 1 and day 7; (3-4) percentage change from day 1 to day 7. Error bars are the range error (n = 2). Red bar is Redgauntlet, yellow bar is Hapil, blue bars are F1 lines.

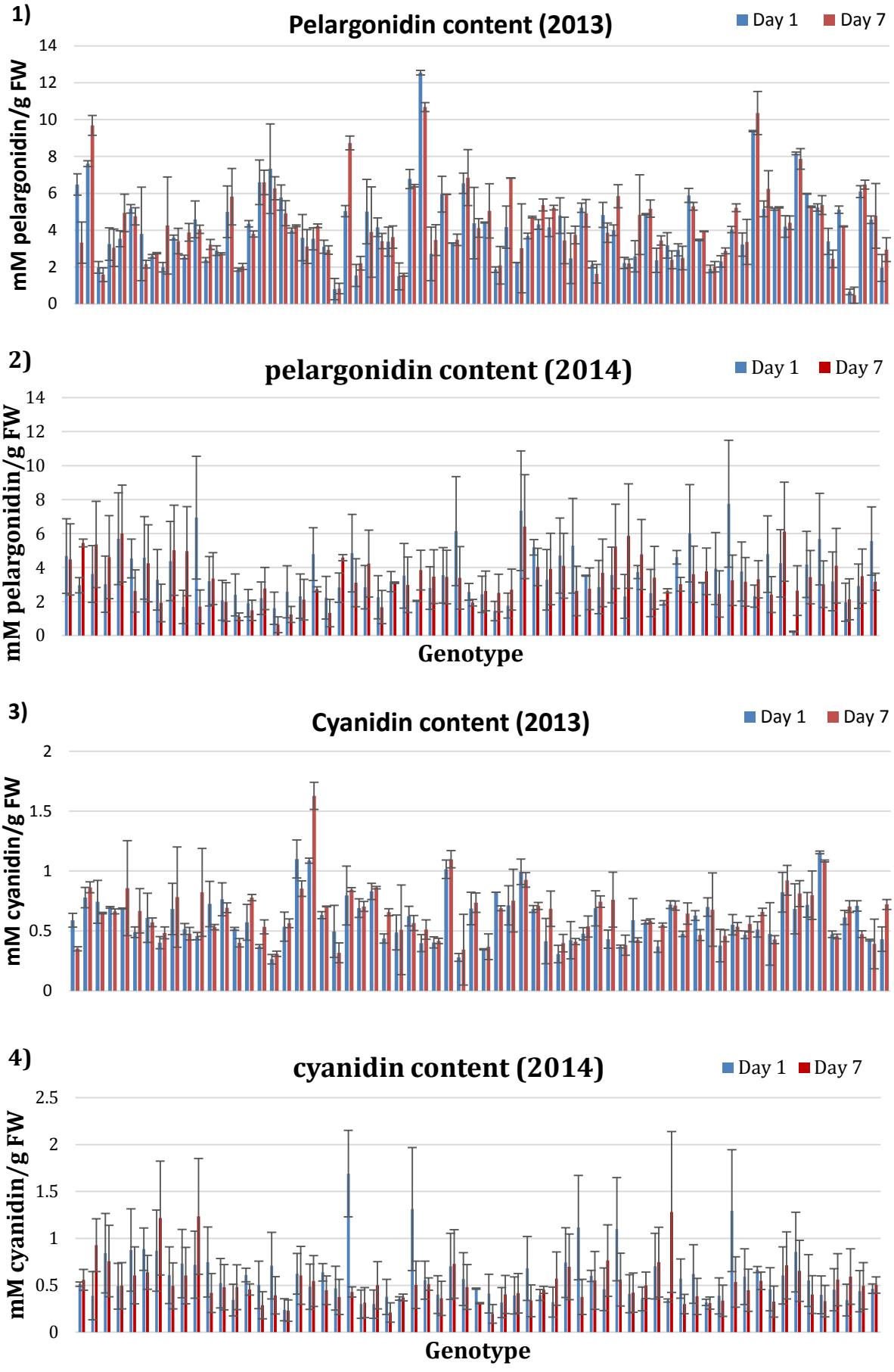
Anthocyanin concentration varies between the parental lines and F1 population over shelf life. However, in season 2013 the change was not statistically significant, while in season 2014 it was significant (Table 3.3 & 3.4). This is in agreement with previous studies by da Silva et al. (2007) and Wang et al. (2002), those reported that the anthocyanin content is varied from year to year or due to different cultivation sites. In RG, the both pelargonidin and cyanidin increased over shelf life storage in both years, which could suggest that the synthesis process of anthocyanins may take place during storage as found previously by Cordenunsi et al. (2005). However, in Hapil, pelargonidin and cyanidin decreased in both

years (Table 3.4). Among the F1 progeny, divergent trends of pelargonidin and cyanidin content were observed where some lines increased while others decreased among the F1 progeny. In season 2013 the majority of the progeny increased in anthocyanin concentration over shelf life, while in season 2014 the majority decreased (Figure 3.9 and Table 3.4). Previous studies reported an increase in the content of phenolic acid, flavonols, and anthocyanins during storage, but this increase was significantly influenced by temperature as the respiratory metabolism rate increased with increasing temperature (Aaby et al., 2012; Cordenunsi et al., 2005; Kalt et al., 1999; Wang and Zheng, 2001). However, Ayala-Zavala et al. (2004) also reported a decrease of the anthocyanin content in strawberry fruits stored at 0 and 5 °C during the first 5 days.

Among the overlapping lines, environmental effect between the two sites was evident on the anthocyanins content (pelargonidin and cyanidin) for only a small number of lines (five lines for pelargonidin and three lines for cyanidin), those performed differently during storage over both years ($p < 0.001$; Table 3.2). For pelargonidin, both parents performed similarly over two years which could suggest that both parents were unlikely to be under the influence of environment for this particular trait. Pelargonidin content of RG increased in both seasons (2013 and 2014), while Hapil decreased (Figure 3.9). Additionally, five overlapping lines including RG001, RG146, RG150, RG162 and RG180 performed differently during storage for the two years which could suggest that

these lines might be influenced by the environmental, while the others performed similarly for both years.

For cyanidin, Hapil seems to be influenced by cultivation sites as it performed differently over two seasons (Figure 3.9). While among the overlapping lines, only two lines might be influenced by the environmental effect those are RG071 and RG125. Although a slightly significant influence of different sites on the anthocyanin content was observed (refer to 3.3.3.1), however the shelf life storage over 7 days showed no significant influence of the shelf life days on anthocyanins content in season 2013. In accordance with previous studies (Aaby et al., 2012; Josuttis et al., 2012), this suggests that anthocyanin profile is more genetically inherited rather than being affected by environmental factors.


1)
Pelargonidin content (2013)
Day 1
Day 7
mM pelargonidin/g FW
14
12
10
8
6
4
2
0
2)
pelargonidin content (2014)
Day 1
Day 7
mM pelargonidin/g FW
14
12
10
8
6
4
2
0
Genotype
3)
Cyanidin content (2013)
Day 1
Day 7
mM cyanidin/g FW
2
1.5
1
0.5
0
4)
cyanidin content (2014)
Day 1
Day 7
mM cyanidin/g FW
2.5
2
1.5
1
0.5
0
Genotype

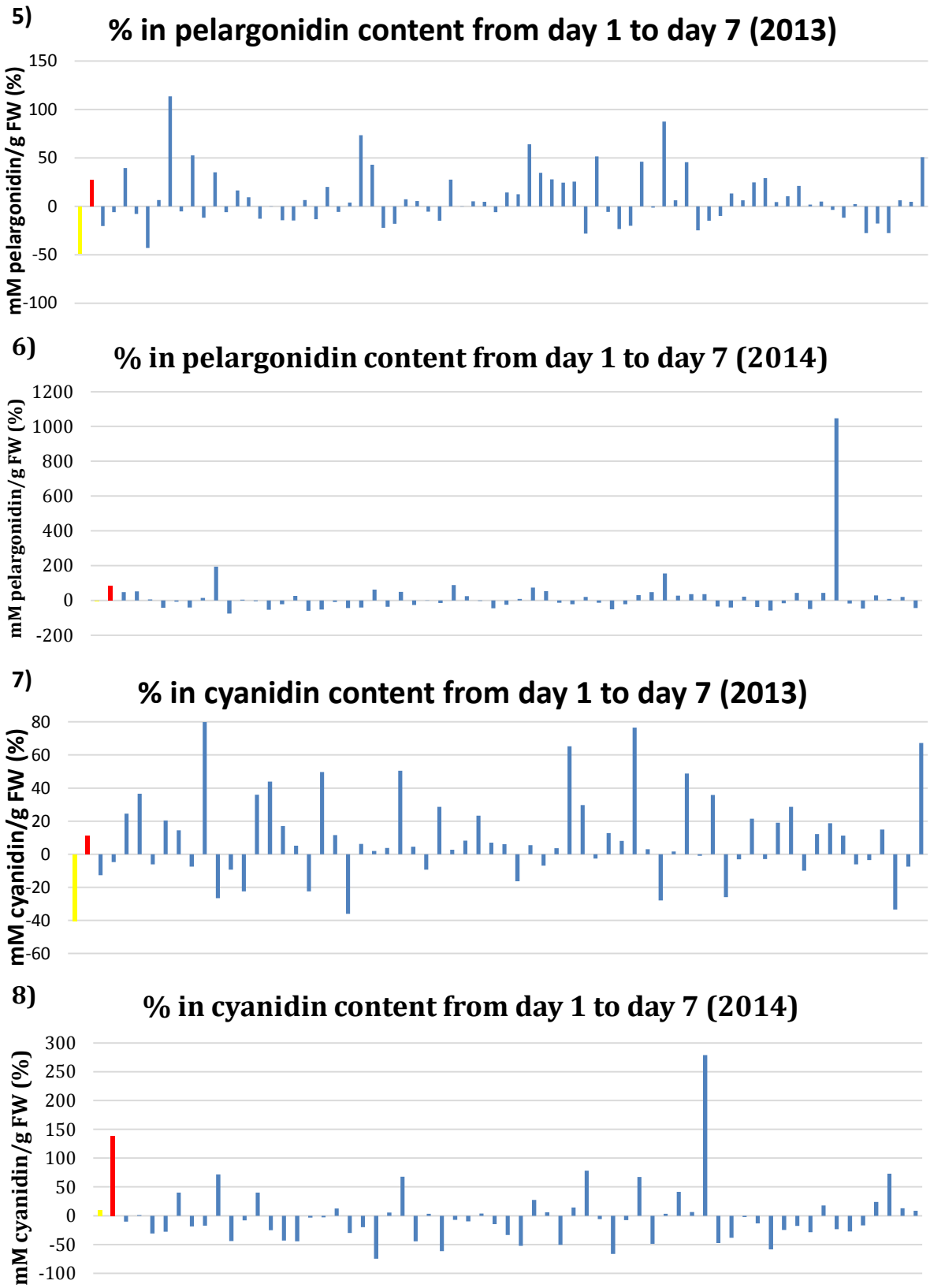


Figure 3.9. Means of anthocyanins between day 1 and day 7 of the F1 progeny plus the parental lines. (1-4) Differences between day 1 and day 7; (5-8) percentage change from day 1 to day 7. Error bars are the range error (n = 2). Red bar is Redgauntlet, yellow bar is Hapil, blue bars are F1 lines.

3.3.5.6 Firmness

Firmness is a key quality parameter in strawberries, since it has a direct relation with fruit ripeness. It has a major economic consequence, soft fruits being more susceptible to bruising (Paz et al., 2008). The firmness of strawberry fruits was monitored over storage of 7 days at 4 °C. As a result of using non-destructive fruits over shelf life in the season 2013, only data of season 2014 were presented. Initial firmness values were above 7.2 N at day 1, however a significant decrease was found in firmness over shelf life for the parental lines as well as most of the offspring lines ($p < 0.001$) (for more details see Table 3.3 & 3.4). As can be seen in Figure 3.10 & table 3.4, 89 % of the lines showed high firmness at day 1 then decreased over day 7, but genotype-specific adverse effects can happen since 11 % of the measured lines were increased.

The decrease was in agreement with the fact that strawberry softening increases with ripening and storage (Ali et al., 2011; Nunes et al., 1995). The decrease in the firmness is due to the degradation of the middle lamella of the cell wall which is regulated by *polygalactunase* enzyme (FaPG1) (Ali et al., 2011; Almenar, E. et al., 2007; Figueroa et al., 2010; Jouki and Dadashpour, 2012; Molina-Hidalgo et al., 2013; Rees et al., 2012; Trainotti et al., 1999; Vicente et al., 2005). It is well-known that the mechanical proprieties of the fruit depend on the cell wall strength and cell-to-cell adhesion.

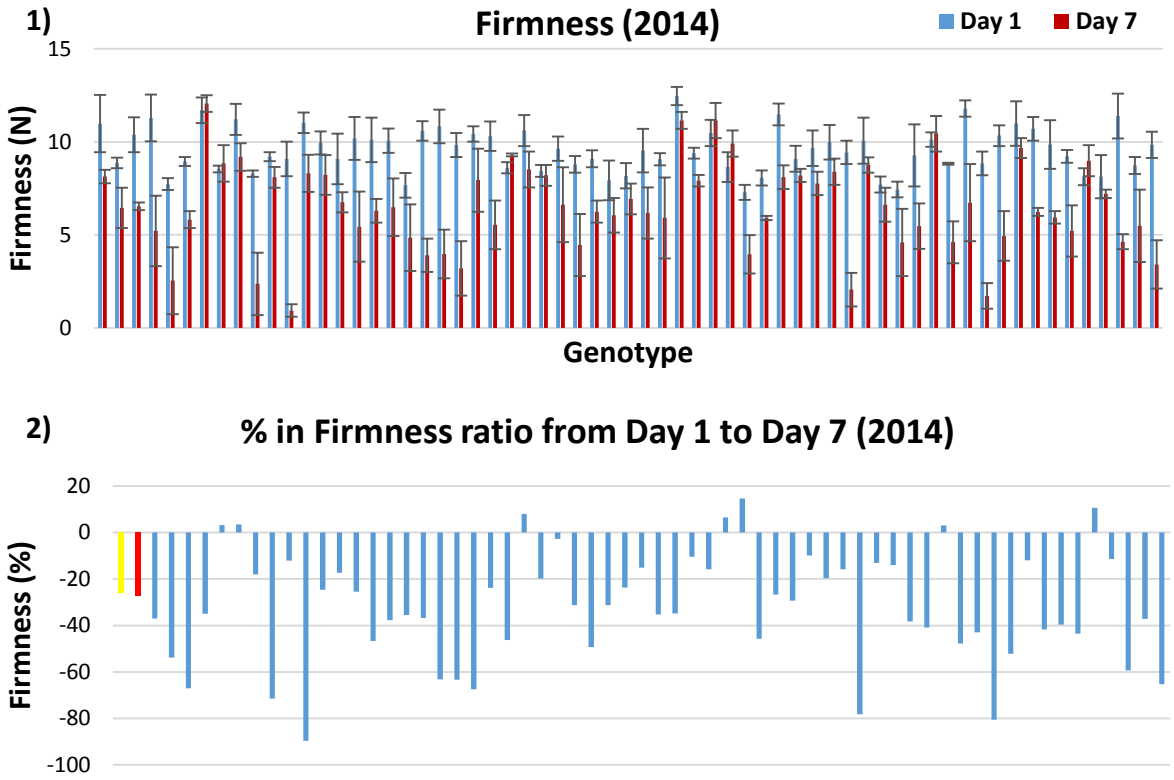


Figure 3.10. Means of firmness between day 1 and day 7 of the F1 progeny plus the parental lines for 2014 data. (1) Differences between day 1 and day 7; (2) percentage change from day 1 to day 7. Error bars are the standard error of means (n = 6). Red bar is Redgauntlet, yellow bar is Hapil, blue bars are F1 lines.

3.3.5.7 Fresh weight

Fresh weight is one of the physical parameters that contribute to the post-harvest quality of strawberries. Storage periods and conditions are key factors in extending strawberry shelf life. Normally, fresh weight loss occurs during fruit storage. As a result of using destructive fruits over shelf life in the season 2013, only data of season 2014 were presented.

100 % of the progeny including the parental lines weighed less at day 7 than day 1 (Figure 3.11, 3.12 and Table 3.5). After 7 days at 4 °C, the highest weight loss

(13.2 %) was obtained for RG175, while the lowest (5.04 %) was obtained for RG149. Such a decrease is expected, as strawberries are very susceptible to water loss that leads to several consequences, one of which is weight reduction which is probably due to fruit transpiration (Miszczak et al., 1995), respiratory process, the transference of humidity and some processes of oxidation (Ayranci and Tunc, 2003).

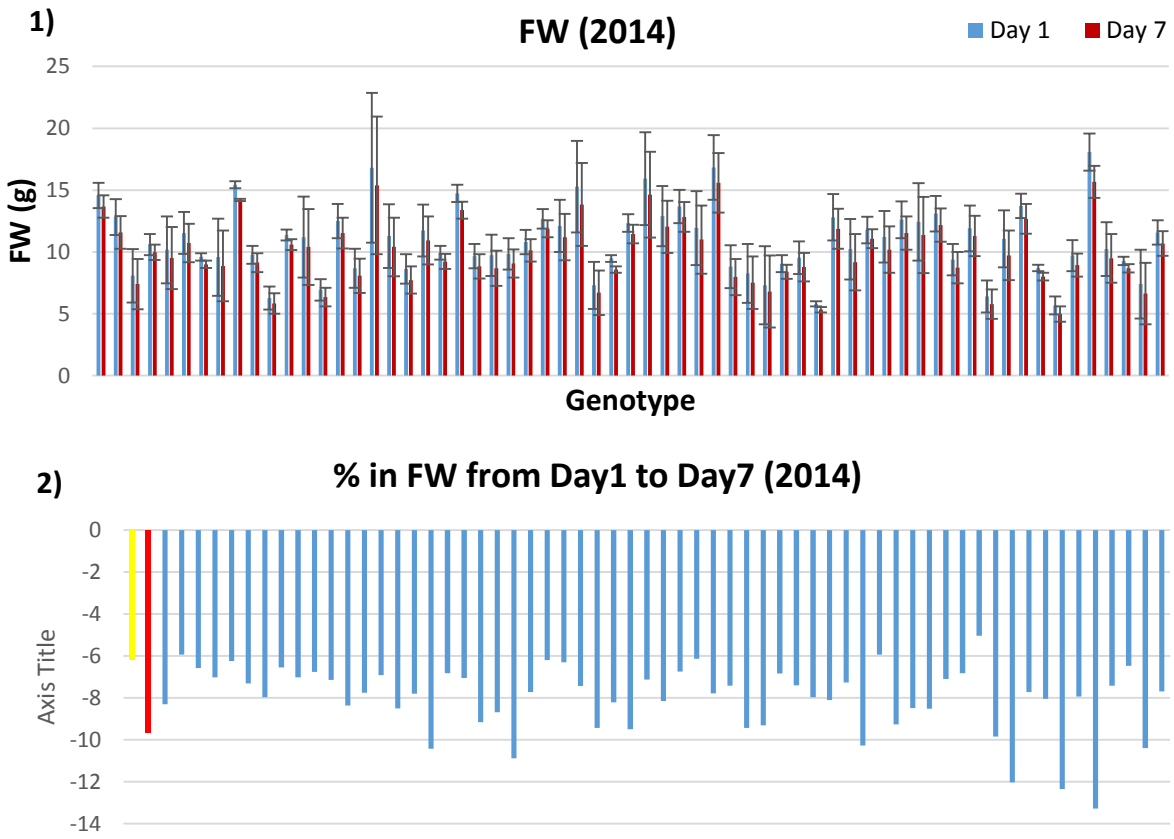


Figure 3.11. Means of FW between day 1 and day 7 of the F1 progeny plus the parental lines for 2014 data, (n = 6). (1) Differences between day 1 and day 7; (2) percentage change from day 1 to day 7. Error bars are the standard error of means (n = 6). Red bar is Redgauntlet, yellow bar is Hapil, blue bars are F1 lines.

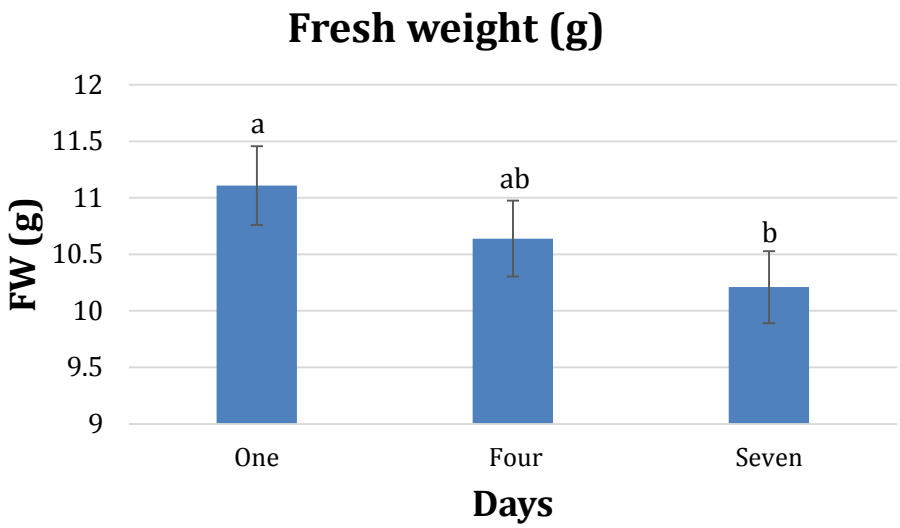


Figure 3.12. Loss of fresh weight in strawberries during storage at 4 °C. Repeated measures on F1 progeny, plus the parental lines, for 2014 data are shown. Error bars are the standard error of means (n = 63). Different letters indicate significant differences ($p < 0.05$). LSD $_{day}$= 0.66.

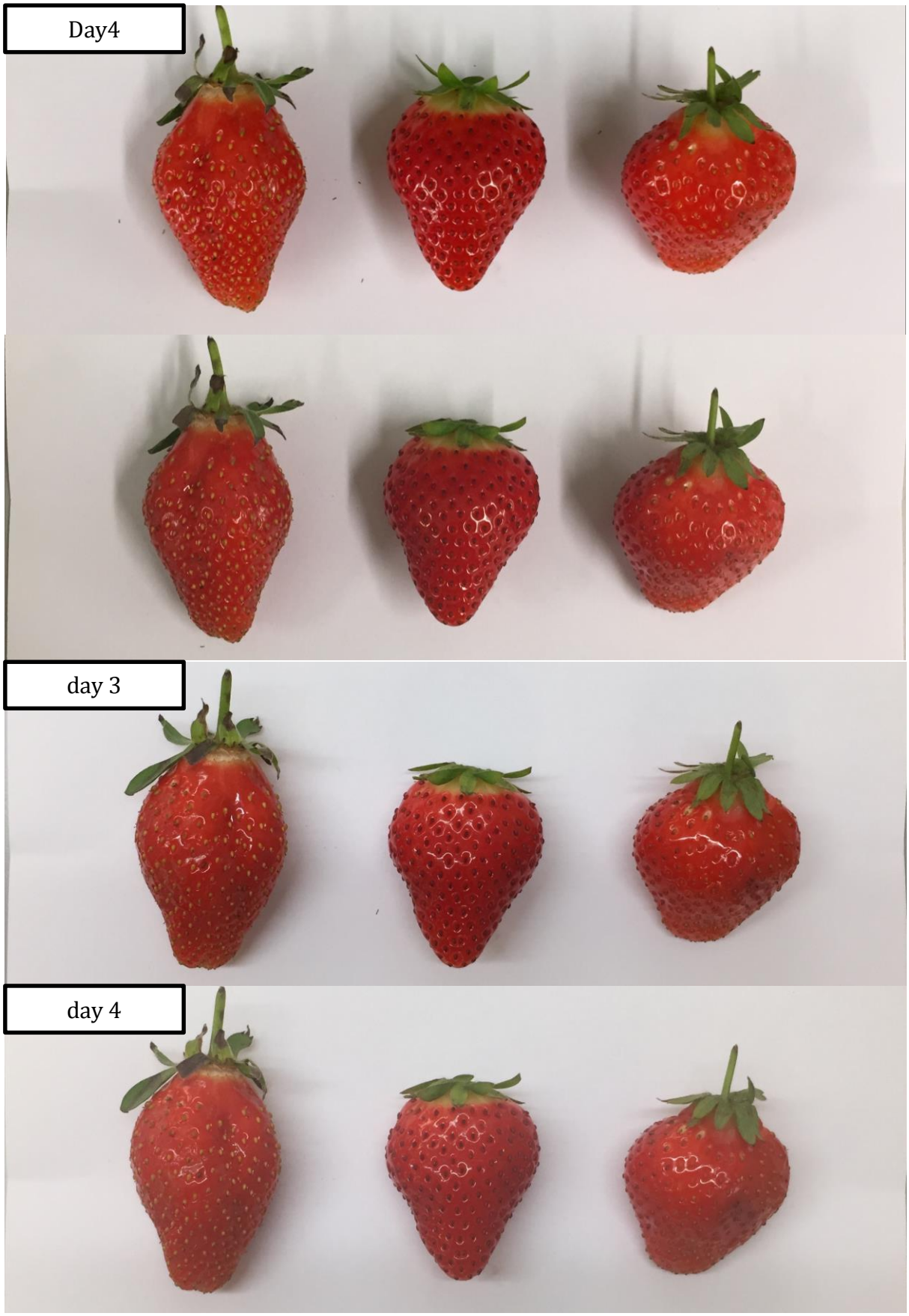
Comparable data reported in the literature showed that the amount of water loss increased during the storage of strawberry at 4 °C for 7 days (Jouki and Dadashpour, 2012). However, they reported 2.9 % as the highest water loss after 7 days that is obviously lower than the highest water loss found in this current study. This might be due to different cultivars being used as well as different measurement practices as their strawberries were packaged with polyethylene during the storage.

3.3.5.8 Visual observation

Strawberry fruits are known to have a very short post-harvest life of 7-9 days if stored in air at 0-5 °C (Ayala-Zavala et al., 2004; Pelayo et al., 2003). This is mainly temperature dependent due to its dramatic influence on biological

reactions and microbial growth (Ayala-Zavala et al., 2004; Li and Kader, 1989). The objective of the following experiment was to monitor the visual appearance of strawberries during shelf life and associate this with the physiological trait of firmness to generate a numerical scale which could subsequently be used to group the lines from the RGxH population into groups of best-worst performing lines.

Three commercial strawberry fruits, supplied in punnets "400 g of UK sweet fruits" (ASDA grower's selection strawberries, Leeds, UK) were stored at 4 °C in order to monitor the visual appearance. The monitoring took place daily (Figure 3.13). The visual quality of strawberry fruit was assessed based on well-known good quality symptoms including fruits with no signs of decay or physiological disorders, infections, dehydration or senescence (Pelayo et al., 2003). The firmness of strawberry fruits was also monitored over storage of 7 days at 4 °C using two destructive fruits for each day.


Day4
day 3
day 4

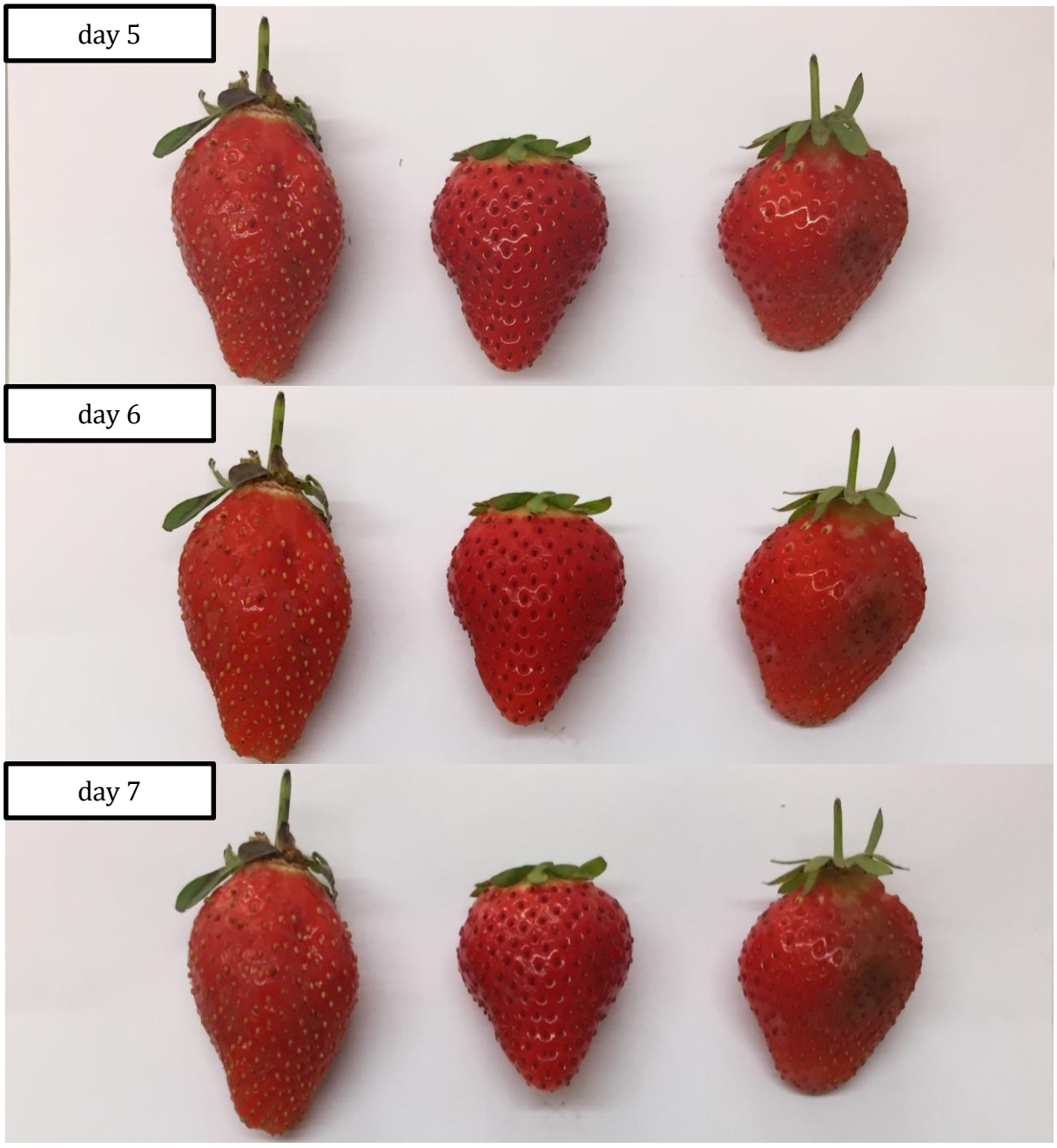


Figure 3.13. Three commercial strawberry fruits, supplied in punnets "400 g of UK sweet fruits" (ASDA grower's selection strawberries, Leeds, UK), were monitored at 4 °C for seven post-harvest days.

An acceptable visual appearance was maintained up to day 4 or 5, depending on the fruit, and then onset of decay incidence was observed on the fruit skin when symptoms of overall poor quality, including bruising, shrivelling, disease

incidence, or off-odour started to appear (Figure 3.14). The severity increased as the number of days progressed. Most probably, this resulted from high water loss during the shelf life storage, as explained above in section 3.3.5.7 where the weight loss was significant at day 7 compared to day 1. This is known to has a negative impact on the physical appearance of the fruit leading to superficial shrivelling and poor colour (Ayala-Zavala et al., 2004; Nunes et al., 1995).

Previous studies on the effect of storage temperature on the overall quality index of strawberry (*Fragaria x ananassa cv. Chandler*) showed that strawberry fruits stored at 5 °C maintained an acceptable quality up to 7 days (Ayala-Zavala et al., 2004). The explanation of the shorter shelf life of our population compared to the findings of Ayala-Zavala et al. (2004) might be attributed to many factors, one of which is cultivar diversity between the two different population. Based on this, the sensory analysis experiment of selected lines was conducted at three post-harvest days up to day 5 (day 1, day 3 and day 5); for more details, see Chapter 5.

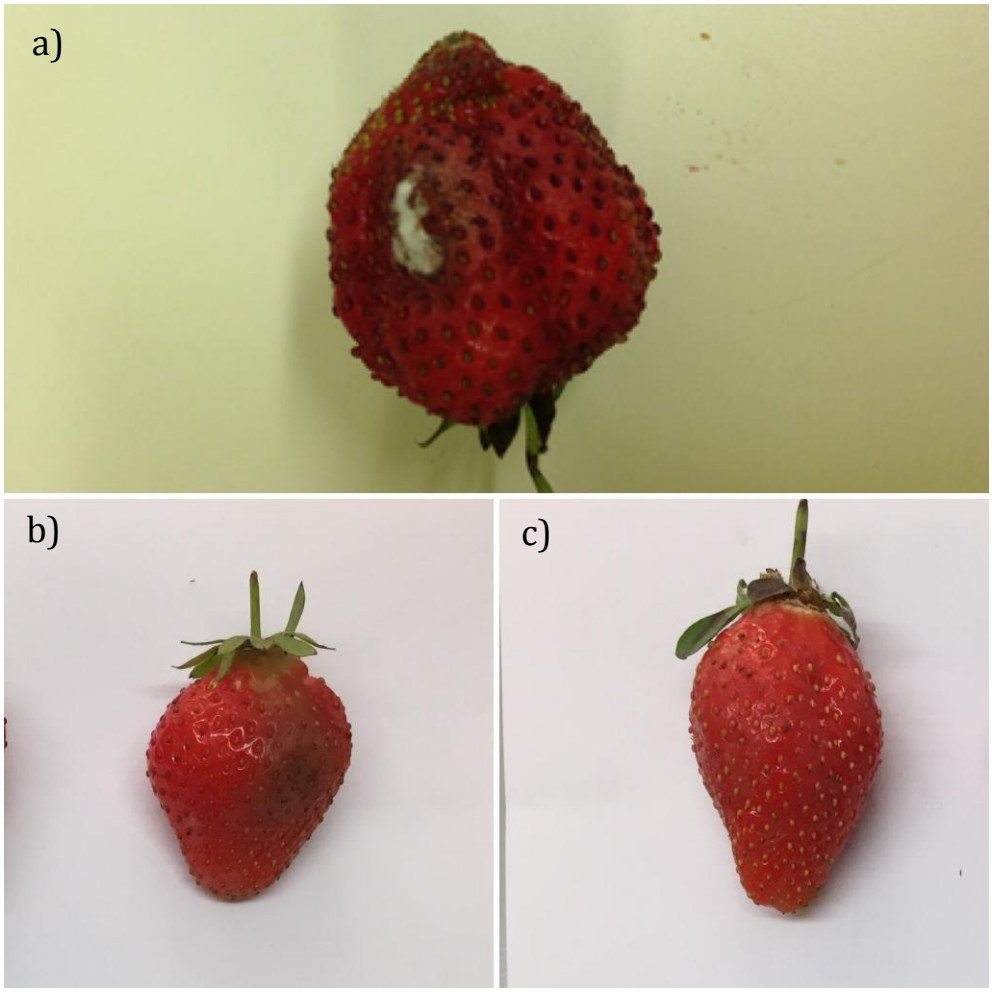


Figure 3.14. Damaged fruits; (a) fruit with grey mould symptoms at day 7, (b) fruit with wet bruise at day 5 and (c) fruit with dry bruise at day 5.

The rate and timing of firmness loss during storage of soft fruits, strawberries as an example, is a key factor to determine fruit quality and post-harvest shelf life. The texture modifications in fruits and vegetables are attributed to many factors including cell wall degradation, enzyme activity, metabolic changes and water content (García et al., 1998). Based on the firmness measurements along with the visual observations of strawberry fruits over storage of 7 days at 4 °C, a numeric firmness scale was generated based on Newton (N) values (≥9 = Firm, 6-8 = Average, ≤6 = Soft; Figure 3.15). Sugar content is used commercially to

determine fruit quality; the recommended range of TSS in strawberries used in commercial practice is 7-12 ºBRIX, depending on the genotype (Ayala-Zavala et al., 2004). In this way, the 63 analysed genotypes of season 2014 were grouped into three best-worst performing classes according to their TSS and firmness data (good, intermediate and poor) as shown in Table 3.5. The classes of best-worst performing were as following; 38 good genotypes, 18 average genotypes and 2 poor genotypes.

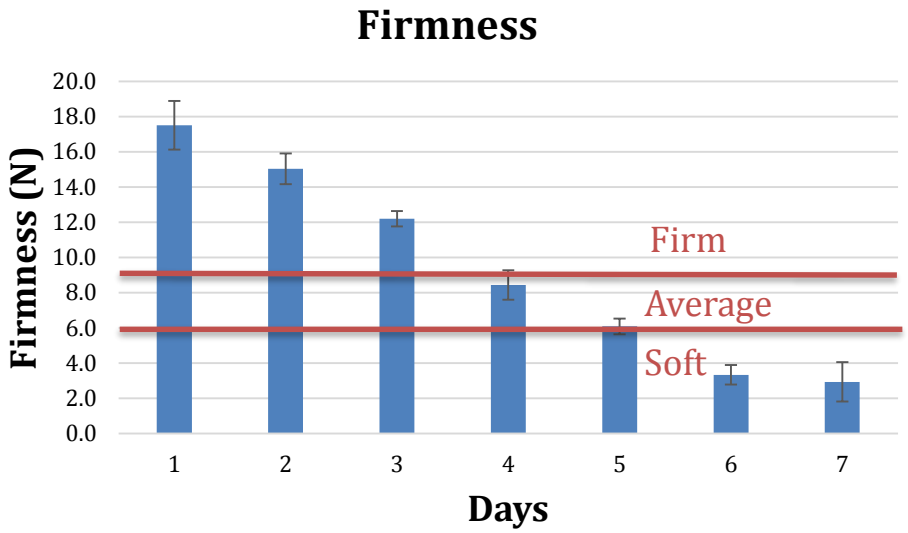


Figure 3.15. Loss of firmness in strawberries during storage at 4 °C. Error bars are the standard error of means. Two commercial strawberry fruits, supplied in punnets with 400 g of UK sweet fruits (ASDA grower's selection strawberries, Leeds, UK) were measured 3 times per a fruit (n = 6) every day over 7 storage days.

Table 3.5. The cluster of best-worst performing of the 63 analysed genotypes of season 2014 according to their TSS and firmness data.

Genotype	Description	Genotype	Description	Genotype	Description
Good		***Good***		***Moderate***	
RG153		RG139		RG029	
RG064		RG177		RG119	- Low TSS - High-average firmness
RG162		RG086		RG055	
RG170		RG011		RG107	
RG002		RG100		RG004	
RG099		RG		RG013	- Gained TSS during storage
RG187	- High TSS - High firmness	RG075		RG006	- Low firmness
RG089		RG038		RG106	
RG097		RG039		RG074	
RG178		RG071	- High TSS - Average firmness	RG010	
RG012		RG149		RG049	- Lost TSS during storage - High firmness
RG145		RG098		RG126	
RG051		RG116		RG077	
RG033		Hapil		RG065	- Lost TSS during storage
RG171		RG117		RG175	- Average firmness
RG088	- Gained TSS during storage	RG023		RG069	
RG167	- High-average firmness	RG125		***Poor***	
RG141		RG067		RG150	- Low TSS - Low firmness
RG001		RG127			

Genotype	Description	Genotype	Description	Genotype	Description
RG020		***Moderate***		***Poor***	
RG026		RG140	- High TSS	RG041	- Lost TSS during storage - Low firmness
RG018		RG146	- Low firmness		
RG043		RG180			

3.4 **Summary**

An investigation of the effect of genotype, storage and two cultivation sites on nutritional and quality traits of the Hapil x RG mapping population was conducted. These results corroborate the dominant role of strawberry genotype (G) in determining quality, however environmental factors (E), including cultivation site and/or storage, as well as G x E still have an influence on most of the measured traits. This is clear in the findings of the current study as some overlapping F1 lines, including the parents had no significant differences between the two sites for several traits. In addition, some traits including TSS (2013), ellagic acid content (2013 and 2014), pelargonidin and cyanidin content (2013) were not significantly influenced by storage. To this point, a number of potential study limitations, including limited number of overlapping lines and different experimental design between the two sites, were identified during the investigation of genotype and environment on strawberry quality. Therefor, the interaction effects found between the genotype (G) and environment (E) for measured quality traits emphasise the importance of evaluating the population during several years and different cultivation sites with standardized experimental design to be able to elucidate the genetic basis of the trait variation observed.

Chapter 4 : Mapping QTL underlying fruit quality traits in an F1 strawberry population

4.1 Introduction

The worldwide annual production of strawberry has been increased in the last years in order to meet the consumer demand (Hummer and Hancock, 2009; Zorrilla-Fontanesi et al., 2011). Therefore, the demand for new strawberry varieties with improved fruit quality traits increased, which means that the breeding programmes are continually looking for methods to improve the efficiency and speed up the process by which new and improved varieties can be produced.

One key target is the development of strawberry varieties with high postharvest quality. The solution could be achieved by the use of marker-assisted breeding, which enables the selection of genotypes which are linked to particular traits of interest, whilst genotypes that do not have the correct genetic composition can be destroyed at the seedling stage making it more cost effective. This approach enables the breeder to make many more crosses per year and select viable progeny extremely early in plant development, thus making better use of glasshouse space and only taking plants to fruiting maturity that are genetically predisposed to expressing the traits of interest.

In order to take a marker assisted breeding approach it is necessary to first develop genetic resources, such as mapping populations, which are supported by

bioinformatics to enable the development of linkage maps. Linkage maps enable DNA polymorphisms that exist between the genomes of parental lines of mapping populations to be placed in an order relative to each other in linkage groups. These polymorphisms can then be scored in the offspring of the parental cross, such that the heredity pattern formed by genetic recombination of the parental genomes by each of the offspring is known. Phenotypic characterization of quality traits is then associated with the genetic polymorphisms in the offspring as the first step towards identifying the underlying candidate genes. The identification of these regions of the genome that are associated with traits of interest are called quantitative trait loci (QTL) and the development of DNA markers linked to the traits of interest will enable plant breeders to use marker-assisted selection breeding. To date, only a limited number of studies exist where a clear marker-trait association for major QTL/genes has been identified in strawberry due to its genome complexity, having eight sets of chromosomes (van Dijk et al., 2014; Zorrilla-Fontanesi et al., 2012).

In this chapter, the Redgauntlet x Hapil (RG x H) population was used, heterozygous cross that segregates for fruit quality, disease resistance and other postharvest traits (Sargent et al., 2009). The aim was: to assess the segregation of the RG x H population for the traits of interest over different length of shelf life storage, to assess the correlation between these traits, and to identify the QTL linked to these traits using single nucleotide polymorphism (SNP) markers. To

achieve this goal: (1) an F1 population derived from the cross of RG x H strawberry parental lines was phenotyped for fruit quality traits during two successive years, (2) a SNP-based genetic linkage map was constructed by Dr Richard Harrison (East Malling Research) using JoinMap 4.0 (Kyazma, NL) (for more details refer to section 2.5.5.2) as shown in the appendix, section 4.1, and (3) data were measured as described in Chapter 2 and associated with the genetic map to map QTL for the traits of interest. It is worth mentioning that, to date, QTL studies of strawberry quality traits have focused on traits measured at the harvest stage, while the majority of fruits reach the consumer only after a period of several days in cold storage and post-harvest storage. Therefore, this study was also carried out to measure the quality traits over different postharvest shelf life points.

4.2 **Materials and methods**

The materials and methods used for the experiments in this chapter are described in detail in Chapter 2.

4.3 **Results and discussion:**

4.3.1 Phenotype distribution and variation within the mapping population

The mapping population was phenotyped for the quality traits of strawberry (fresh weight, firmness, total soluble solids (TSS), titratable acidity (TA), phenolic content and colour) measured over three post-harvest days in two sequential seasons at two separate field sites, in East Malling Research and University of Reading, in 2013 and 2014, respectively (for more evidence see Chapter 2; section

2.3, and Chapter 3; sections 3.3.1 and 3.3.4). Transgressive segregation could be seen in all traits in which some F1 individuals showed extreme values that were both higher and lower extremes relative to the two parent lines (Figure 4.1 & 4.2; for more results, refer to Chapter 3; Table 3.3).

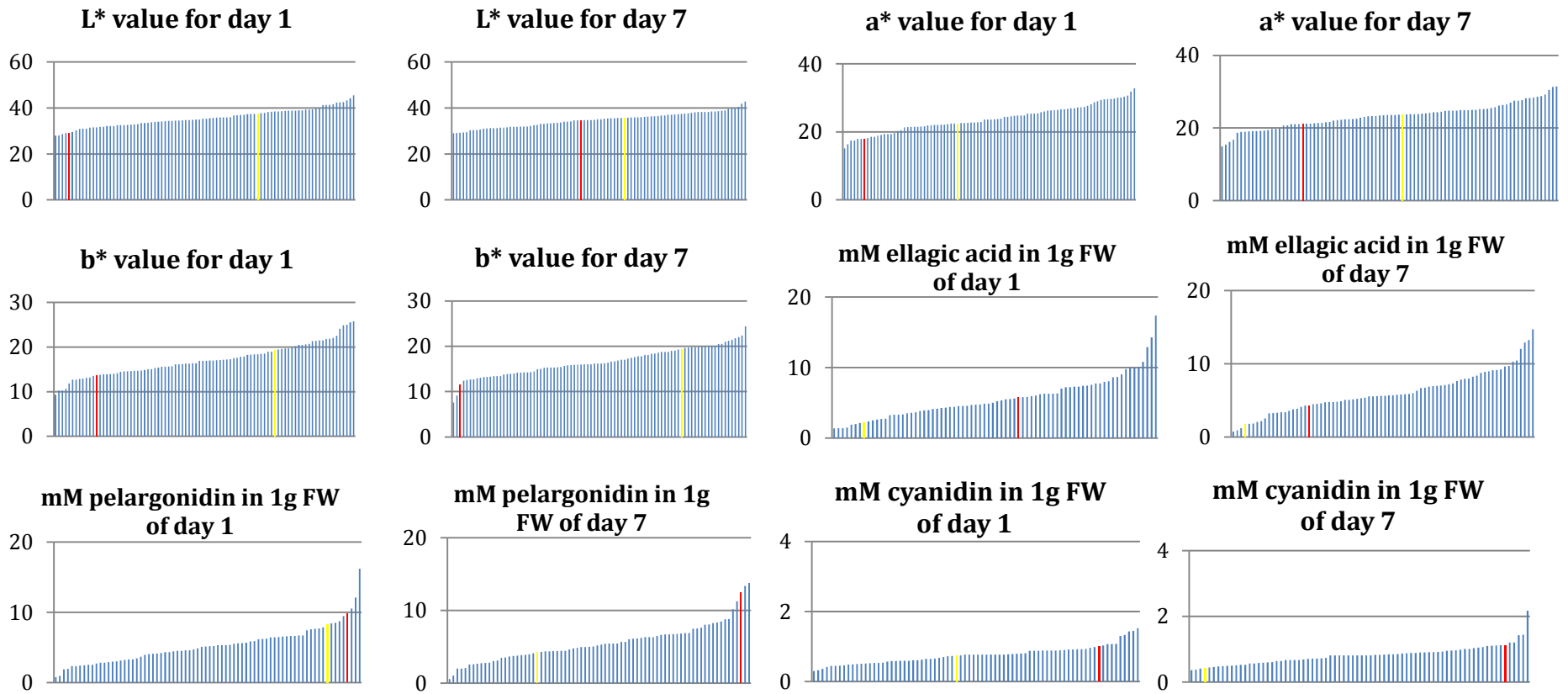
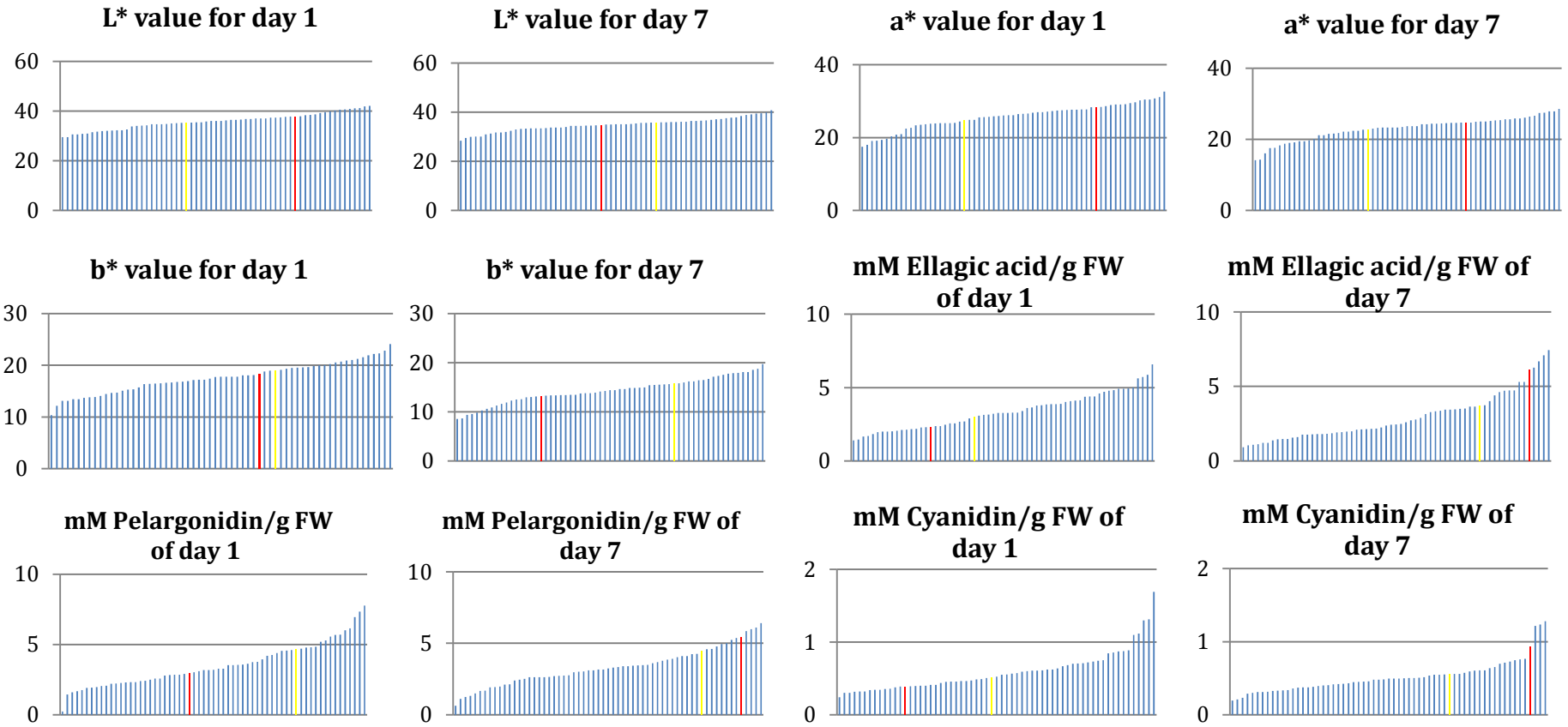


Figure 4.1. Segregation across the population for different traits of 2013 data. Values are the means of measurements generated by ANOVA, n=4 for colour measurements (L*, a*, b*) and n=2 for phenolic acid contents. SEMs: $L^*_{day\ 1}$=0.41, $L^*_{day\ 7}$=0.33, $a^*_{day\ 1}$=0.41, $a^*_{day\ 7}$=0.35, $b^*_{day\ 1}$=0.37, $b^*_{day\ 7}$=0.32, $EA_{day\ 1}$=0.24, $EA_{day\ 7}$=0.23, $Pel_{day\ 1}$=0.22, $Pel_{day\ 7}$=0.23, $Cya_{day\ 1}$=0.024, $Cya_{day\ 7}$=0.027. Red column is RG and yellow column is Hapil, F1 progeny genotypes are shown in blue.


L* value for day 1
60
40
20
0
L* value for day 7
60
40
20
0
a* value for day 1
40
20
0
a* value for day 7
40
20
0
b* value for day 1
30
20
10
0
b* value for day 7
30
20
10
0
mM Ellagic acid/g FW of day 1
10
5
0
mM Ellagic acid/g FW of day 7
10
5
0
mM Pelargonidin/g FW of day 1
10
5
0
mM Pelargonidin/g FW of day 7
10
5
0
mM Cyanidin/g FW of day 1
2
1
0
mM Cyanidin/g FW of day 7
2
1
0

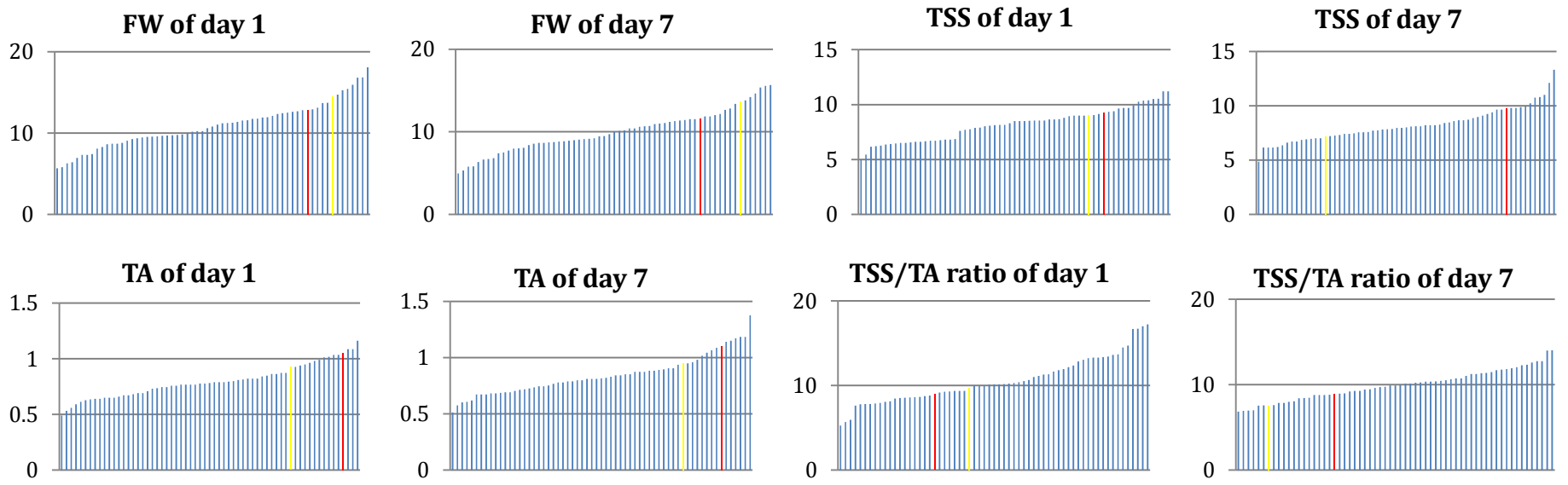


Figure 4.2. **Segregation across the population for different traits of 2014 data. Values are the means of measurements generated by ANOVA, n=6 for FW and colour measurements (L*, a*, b*), n=2 for TSS and TA, and n=2 for phenolic acid contents. SEMs: $L^*_{day\ 1}$=0.33, $L^*_{day\ 7}$=0.27, $a^*_{day\ 1}$=0.43, $a^*_{day\ 7}$=0.40, $b^*_{day\ 1}$=0.36, $b^*_{day\ 7}$=0.32, $EA_{day\ 1}$=0.15, $EA_{day\ 7}$=0.19, $Pel_{day\ 1}$=0.19, $Pel_{day\ 7}$=0.16, $Cya_{day\ 1}$=0.03, $Cya_{day\ 7}$=0.027, $TSS_{day\ 1}$=0.18, $TSS_{day\ 7}$=0.19, $TA_{day\ 1}$=0.02, $TA_{day\ 7}$=0.02, $ratio_{day\ 1}$=0.33, $ratio_{day\ 7}$=0.22. Red column is RG and yellow column is Hapil, F1 progeny genotypes are shown in blue.**

For all measured traits, extreme lines were observed which is commonly detected in all population studies and often observed in populations derived from intraspecific crosses (DeVicente and Tanksley, 1993; Rieseberg et al., 1999). Transgressive segregation was reported in strawberry fruit of an octoploid strawberry for agronomical and quality traits including, but not limited to, FW, firmness, colour, sugar, acid, anthocyanin content and yield (Lerceteau-Kohler et al., 2012; Zorrilla-Fontanesi et al., 2011). This phenomenon is probably due to the complementary gene action as an effect of inherited parental alleles on both directions of the trait as found previously by (DeVicente and Tanksley, 1993). The occurrence of transgression in strawberry might be stronger due to the large number of alleles in these polyploid species that may act epistatically to each other and are therefore possibly responsible for the formation of extreme lines (Coelho et al., 2007; Lerceteau-Kohler et al., 2012).

For breeding programmes, transgressive segregation is significant with respect to crop improvement and indicates the extent of genetic diversity in the population which suggests its suitability for detecting QTL. The genetic variation within a population can lead to phenotypic variation as a result of new pairing of alleles in the different lines arising from the F1 cross. This is in alignment with the current data analysis of the variability among the F1 progeny where the transgressive segregation was evident. This variation could be a result of different factors, one of which is a genetic variation that was likely caused by the high heterozygosity

of the parents leading to new allele combinations in the offspring. Although some observed transgressive phenotypes have no practical value, showing such variation shows that the population has enough diversity to potentially identify some QTL linked with the measured traits.

It is important to study the phenotypic data to ensure the normality of the data and the segregation among the population. The success of QTL mapping depends crucially on the integrity of the data, one main factor of the integrity is a normal distribution of the phenotype trait data. As the original data exhibited non-normal distribution, alternative actions have been taken to normalise the distribution of the data by the log-transformation of the data using the excel function. The transformed data of the 51 traits showed a continuous variation among the population between the measured traits and normal distribution was also observed for most of the traits (Figure 4.3 & 4.4).

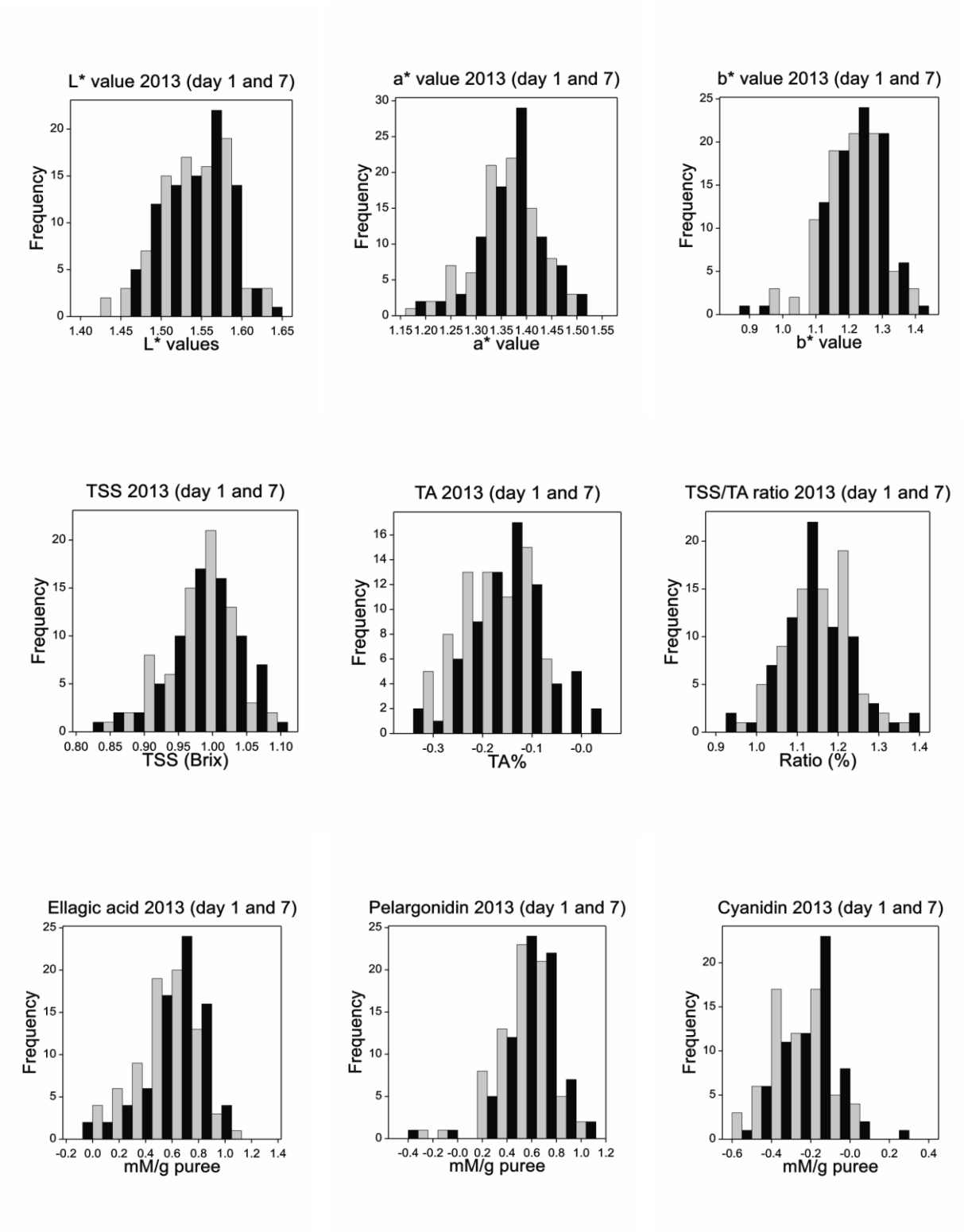


Figure 4.3. Distribution of the values of traits phenotyped in 2013. Values are of log-transformed data of the means of measurements generated by ANOVA. L*, a* and b* values are the colour parameters, TSS is total soluble solids, TA is titratable acidity. The light grey bars are day 1 values and the black bars are day 7 values.

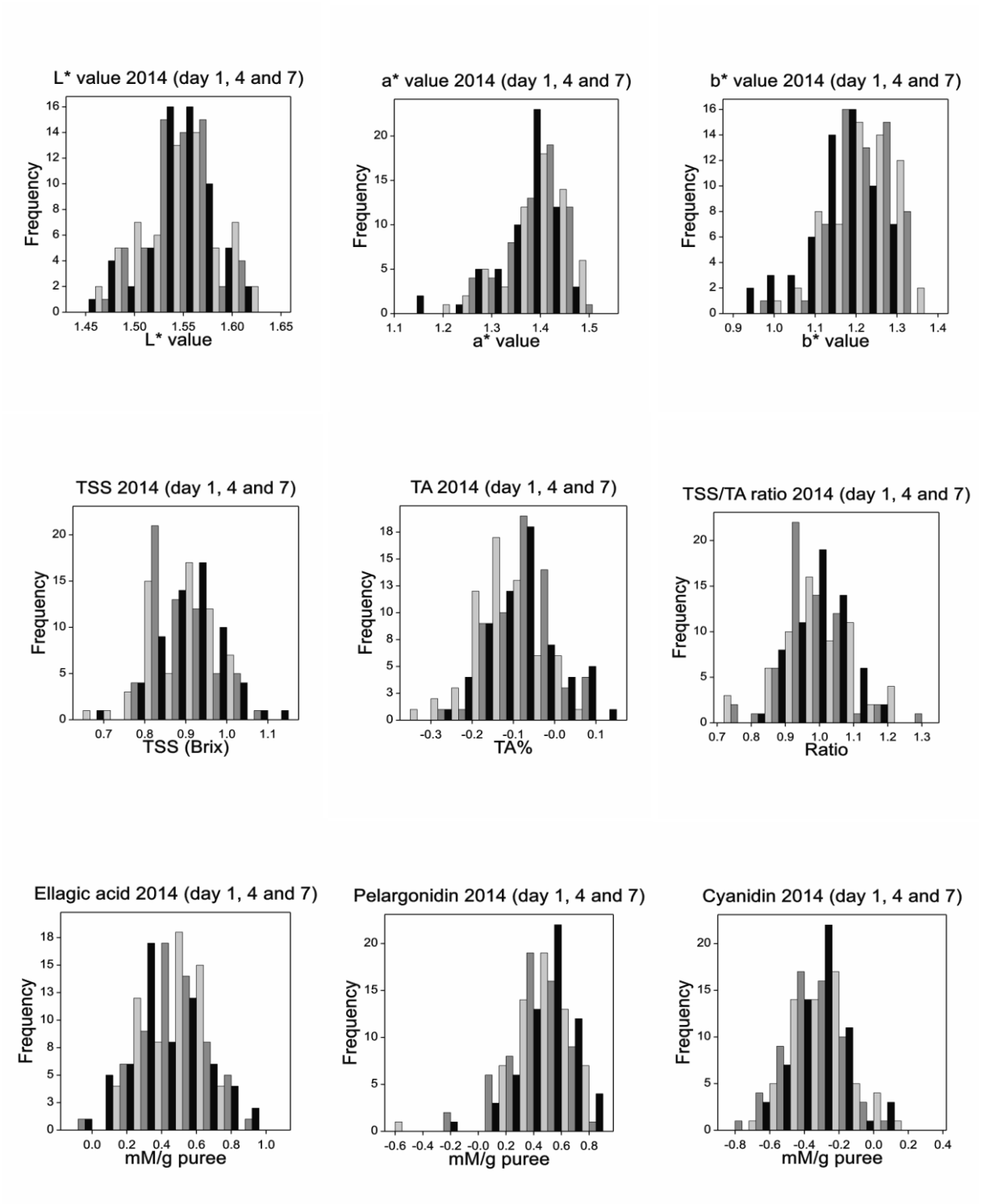


Figure 4.4. Distribution of the values of traits phenotyped in 2014. Values are of log-transformed data of the means of measurements generated by ANOVA. L*, a* and b* values are the colour parameters, TSS is total soluble solids, TA is titratable acidity. The light grey bars are day 1 values, the dark grey bars are day 4 values, and the black bars are day 7 values.

Broad-sense heritability (H^2), the ratio of total genetic variance to total phenotypic variance, ranged from 0.24 for (the colour value of redness-greenness; a-7-13) to 0.96 for (Pel-4-14) (Table 4.3 & 4.4). For 18 out of 51 traits, heritability displayed high values ($H^2 > 0.5$), suggesting that the variation in these particular traits is due to variation in genetic factors (Wray and Visscher, 2008). Additionally, 12 of the 18 analysed traits, including TSS, TA, TSS/TA ratio, and phenolic compounds (ellagic acid and pelargonidin), showed high values ($H^2 > 0.7$). Such high values therefore suggest that these phytochemicals are strongly controlled by genetic factors (Wray and Visscher, 2008). However, low heritability values ($H^2 < 0.3$) were also observed for two colour parameters measured in season 2013 (L-7-13 and a-7-13). On top of these findings of the distribution, segregation and heritability of the population, ANOVAs showed that there was significant genetic variability for all the above traits in this population (for more evidence refer to section 3.3.4), thus permitting further QTL analysis.

4.3.2 Correlation among the traits

Correlations between the traits in each field trial were investigated to determine the extent to which traits are correlated with each other, using Pearson's correlation coefficient analysis. Due to the large dataset collected over two years, only significant correlations are shown (Table 4.1 & 4.2). Correlations between the three colour parameters (L*, a* and b* values), where L* value is lightness, a* value is redness-greenness, and b* value is yellowness-blueness, were

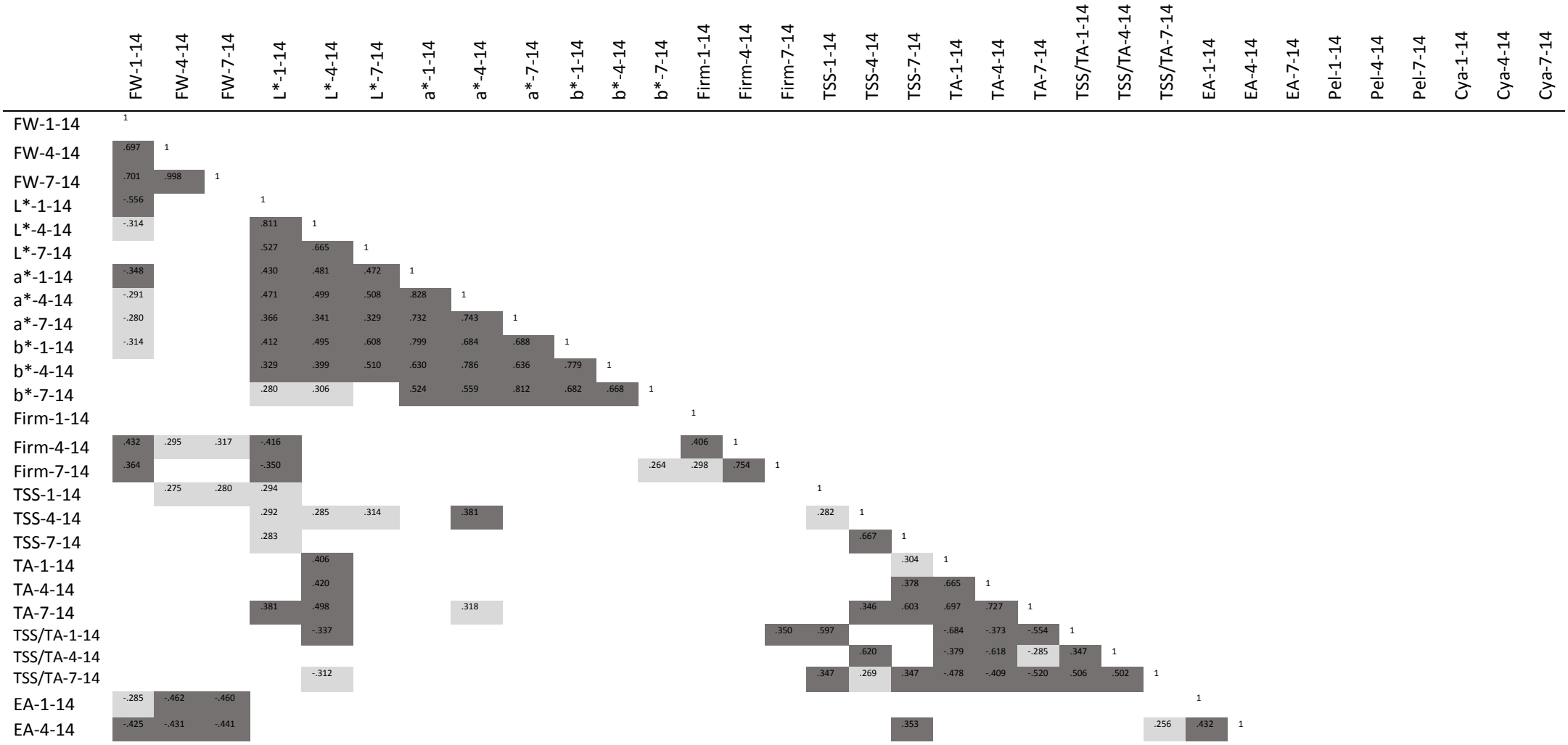
positively significant in each two years (different P values depending on the trait and on the year). In addition, a highly negative correlation ($p \leq 0.01$) was also found between colour parameters (L*, a* and b* values) and anthocyanin content (pelargonidin and cyanidin), the main pigments contributing to strawberry redness, for both years and over all post-harvest days. This is in agreement with previously reported studies in strawberry (Lerceteau-Kohler et al., 2012; Zorrilla-Fontanesi et al., 2011) and cherry (Gonçalves et al., 2007; Kasım et al., 2011; Viljevac et al., 2012) those found a negative correlations between all colour readings and anthocyanins. This suggest that the decrease in colour parameters L*, a* and b* is probably due to the increase in anthocyanin content of the fruit which leads the fruits to be darker at the end of the shelf life, since lower colour parameters indicate overall dark fruit. By contrast, no correlation was found between the surface colour measurements and anthocyanins in strawberry (Ordidge et al., 2012). This latter study was confined to total anthocyanin content, when change of colour was poorly correlated the total anthocyanins. However, previous report from strawberry pulp from six cultivars shown that the a* value correlated with both pelargonidin-glucoside and the total pelargonidin content (Skupień and Oszmiański, 2004).

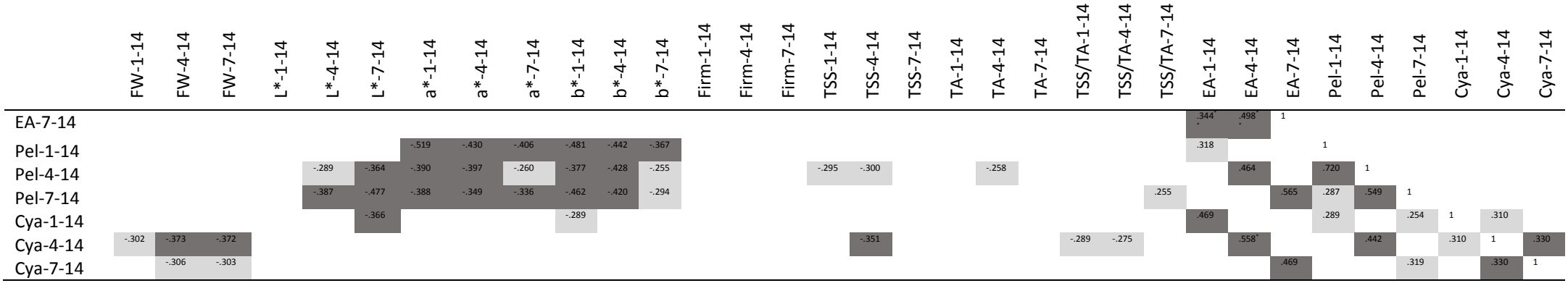
Table 4.1. Pearson's correlations for the analysed traits of the RG × Hapil F1 population (2013 data).

	L*-1-13	L*-7-13	a*-1-13	a*-7-13	b*-1-13	b*-7-13	TSS-1-13	TSS-7-13	TA-1-13	TA-7-13	TSS/TA -1-13	TSS/TA -7-13	EA -1-13	EA -7-13	Pel-1-13	Pel-7-13	Cya-1-13	Cya-7-13
L*-1-13	1																	
L*-7-13	.652**	1																
a*-1-13	.630**	.500**	1															
a*-7-13	.556**	.573**	.819**	1														
b*-1-13	.631**	.569**	.791**	.652**	1													
b*-7-13	.627**	.546**	.671**	.680**	.650**	1												
TSS-1-13							1											
TSS-7-13							.701**	1										
TA-1-13	.376**	.251*	.251*		.360**	.311**		.235*	1									
TA-7-13		.238*			.270*				.701**	1								
TSS/TA -1-13	-.330**				-.286*		.495**		-.788**	-.562**	1							
TSS/TA -7-13							.374**	.531**	-.422**	-.743**	.607**	1						
EA-1-13			-.255*				-.311*		-.330**	-.270*			1					
EA-7-13									-.255*				.804**	1				
Pel-1-13	-.313**	-.370**	-.527**	-.424**	-.500**	-.319**			-.409**	-.294*	.261*		.552**	.472**	1			
Pel-7-13	-.330**	-.345**	-.531**	-.455**	-.517**	-.375**			-.345**	-.327**	.269*	.258*	.556**	.576**	.876**	1		
Cya-1-13							-.397**						.610**	.524**	.460**	.382**	1	
Cya-7-13													.454**	.700**	.365**	.436**	.777**	1

****** indicates significant at P ≤ 0.01 (2-tailed; dark grey), ***** indicates significant at P ≤ 0.05 (grey). Only significant correlations are shown.

Table 4.2. Pearson's correlations for the analysed traits of the RG × Hapil F1 population (2014 data).





****** indicates significant at P ≤ 0.01 (2-tailed; dark grey), ***** indicates significant at P ≤ 0.05 (dark grey). Only significant correlations are shown. Codes were used for all quality traits refer to "trait-day-year".

Among the first year data (season 2013), the strongest correlation ($p \leq 0.01$) was observed between a*-1-13 (value is redness-greenness) and Pel-7-13 (anthocyanins) (-0.531). A few negative correlations were significant for both seasons (2013 and 2014) between titratable acidity (TA) and TSS/TA ratio and also between total soluble solids (TSS) and anthocyanin content (pelargonidin and cyanidin). For the second year data (season 2014), fresh weight (FW) was negatively significantly correlated with ellagic acid, anthocyanins (cyanidin), and positively significantly correlated with firmness, suggesting that the larger the fruit size, the lower the polyphenols content is. Previous study reported that the content of total phenolic and ellagic acid were lowest in primary fruits, which are often larger in size (Anttonen et al., 2006). This is in agreement with the fact that as the fruit is bigger, the resources are allocated for the growth which enhance the protein synthesis, which eventually lead to the lower substrate availability for phenylalanine ammonia lyase (PAL) and thus reduction in the phenolic content (Anttonen et al., 2006). It might be also due to the dilution factor caused by increased biomass (Anttonen et al., 2006).

Correlations between fruit quality traits obtained for the RG x H population are in agreement with previously reported correlations in other varieties of strawberry (Lerceteau-Kohler et al., 2012; Shaw, 1988; Zorrilla-Fontanesi et al., 2011). For example, a positive correlation was detected between TA and TSS in some shelf life days for both years (Table 4.1 & 4.2), which is similar to what was reported

earlier by Shaw (1988), Zorrilla-Fontanesi et al. (2011), and Lerceteau-Kohler et al. (2012) for the correlation between pH and TA with TSS and sugar compounds (fructose, sucrose, and glucose). Moreover, a negative correlation was detected between TSS and anthocyanins in some shelf life days for both years (Table 4.1 & 4.2). It is well-known that sugars are the initial precursor of the anthocyanin biosynthesis during ripening (Hrazdina et al., 1984; Ruhnan and Forkmann, 1988; Teusch et al., 1987), therefore, such correlations between these two traits are expected.

4.3.3 QTL analysis

4.3.3.1 QTL detection

A genetic map containing 3933 SNP markers was used for composite interval mapping (IM) and multiple QTL mapping (MQM) analysis. First, the number of SNPs had to be reduced to 523 SNPs distributed over the 28 LGs, due to the computational problem that MapQTL programme could not cope with the marker overload (for more details about the map, see section 2.5.5.1). The total genome size was 2626 centimorgan (cM) and the average interval was 5 cM between markers. QTL were analysed using log-transformed data of 51 quality traits (11 post-harvest traits over different shelf life days) for each year separately. Despite the genetic complexity of the strawberry genome, a total of 47 QTL were detected using interval mapping (IM) in combination with restricted multiple QTL mapping (rMQM) (Van Ooijen, 2006), across 22 LGs out of the 28 LGs, for 24

traits over two consecutive years (2013 and 2014) (Table 4.3 & 4.4), table of cofactors are shown in the appendix, sections 4.4. Between one QTL (for TSS/TA-1-13, a-7-13, Pel-1-14, Firmness-4-14, L-4-14, a-4-14, TSS-4-14, and TA-4-14) and five QTL (for TSS-7-14) have been identified per trait, with the phenotypic variation (R^2) explained by each QTL ranging from 7.6 % (for TSS-7-14 on 48.609 cM of LG4A in 2014) and 38.2 % (for TSS-7-14 on 24.383 cM of LG5).

Table 4.3. QTL detected for five quality traits of year 1 (season 2013) in the RG x Hapil population based on IM model mapping followed by MQM, and rMQM.

Trait	LG	Position (cM)	Locus	LOD[a]	Explained variance (%)[b]	H^2	Parental effect
TSS/TA-1-13	LG3A	68.553	AX-89823927:ph3	4.06	19.3	0.76	RG
L-1-13	LG4B	70.412	AX-89791332:nmh	5.26	22.9	0.56	Hapil
	LG6B	6.352	AX-89915259:nmh	3.27	13.4		RG
L-7-13	LG1A	37.129	AX-89780485:nmh	4.52	20.9	0.25	Hapil
	LG2B	59.42	AX-89880621:ph3	3.34	14.1		RG
a-7-13	LG1A	32.217	AX-89875633:nmh	4.15	19.9	0.24	RG
TSS/TA-7-13	LG4B	60.282	AX-89788864:nmh	4.54	22.8	0.66	RG
	LG3C	42.698	AX-89784703:nmh	3.56	17.3		Hapil
8 QTL (5 traits)							

LOD threshold of 3.2 (Ooijen, 1999) was used for all traits and groups to identify potential QTL. **[a]** LOD above the threshold, **[b]** Percentage of total phenotypic variation explained by the QTL. Codes were used for all quality traits refer to "trait-day-year".

Table 4.4. QTL detected for 19 quality traits of year 2 (season 2014) in the RG x Hapil population based on IM model mapping followed by MQM, and rMQM.

Trait	LG	Position (cM)	Locus	LOD[a]	Explained variance (%)[b]	H^2	Parental effect
FW-1-14	LG3A	83.392	AX-89784929:nmh	5.21	28.2		RG
	LG6B	52.054	AX-89849864:ph3	3.25	16.3	0.41	Hapil
	LG1B	15.703	AX-89904113:ph3	3.24	12.7		Hapil
Cya-1-14	LG1D	17.367	AX-89875407:nmh	4.66	24.9	0.53	RG
	LG1A	15.33	AX-89816729:nmh	3.28	16.6		Hapil
Pel-1-14	LG4B	0	AX-89788656:nmh	4.38	28.9	0.95	RG
EA-1-14	LG6C	25.848	AX-89850346:nmh	4.07	21.3	0.79	RG
	LG4D	48.831	AX-89887216:nmh	3.38	17.2		RG
TSS/TA-1-14	LG3A	89.707	AX-89785116:nmh	5.4	21.1		RG
	LG6A	37.024	AX-89899527:nmh	4.19	15.6		Hapil
	LG3D	40.511	AX-89784364:nmh	3.91	14.4	0.90	RG
	LG7B	23.512	AX-89800314:nmh	3.59	13.1		Hapil
	LG7A[c]	0.18	AX-89872084:nmh	0.61	1.9		RG
FW-4-14	LG3A	83.392	AX-89784929:nmh	7.97	**37.5**		RG
	LG6B	52.054	AX-89849864:ph3	4.09	16.5	0.42	Hapil
	LG1B	15.703	AX-89904113:ph3	3.26	12.7		Hapil
Firmness-4-14	LG6C	12.103	AX-89899781:nmh	4.1	16.4	0.49	Hapil
L-4-14	LG6B	89.744	AX-89915591:ph3	3.25	21	0.55	RG
a-4-14	LG1B	74.025	AX-89779306:ph3	3.44	22.1	0.57	RG
TSS-4-14	LG1A	4.327	AX-89779683:nmh	3.42	22.8	0.94	Hapil
TA-4-14	LG2C	54.864	AX-89806659:nmh	3.73	24.6	0.94	RG
TSS/TA-4-14	LG7A	0.18	AX-89872084:nmh	5.11	19.1		RG
	LG5B	22.768	AX-89861737:ph3	4.78	17.6	0.95	Hapil
	LG6A	30.635	AX-89842577:ph3	4.68	17.2		Hapil
Pel-4-14	LG2B	81.844	AX-89874909:ph3	3.76	24.7	0.96	Hapil
FW-7-14	LG3A	83.392	AX-89784929:nmh	7.97	37.8		RG
	LG6B	52.054	AX-89849864:ph3	3.92	15.8	0.41	Hapil
	LG1B	15.703	AX-89904113:ph3	3.36	13.2		Hapil
EA-7-14	LG6A	99.005	AX-89797034:ph3	5.89	25.4		Hapil
	LG4C	64.537	AX-89781839:ph3	3.43	13.4	0.90	Hapil
	LG2A	38.335	AX-89782715:nmh	3.24	12.5		RG

Trait	LG	Position (cM)	Locus	LOD[a]	Explained variance (%)[b]	H^2	Parental effect
Pel-7-14	LG7D	18.517	AX-89800941:nmh	3.32	22.2	0.85	RG
TSS-7-14	LG5A	24.383	AX-89893282:ph3	12.98	38.2		Hapil
	LG7D	45.015	AX-89802341:ph3	10.32	27.1		Hapil
	LG6C	95.604	AX-89897268:nmh	7.13	16.4	0.94	Hapil
	LG2A	95.175	AX-89877249:nmh	4.23	8.6		RG
	LG4A	48.608	AX-89790195:nmh	3.78	7.6		Hapil
TA-7-14	LG5C	19.34	AX-89874899:nmh	4.5	25.4	0.87	RG
TSS/TA-7-14	LG7A	0.18	AX-89872084:nmh	4.59	29.3	0.86	RG
39 QTL (19 traits)							

LOD threshold of 3.2 (Ooijen, 1999) was used for all traits and groups to identify potential QTL. **[a]** LOD above the threshold, **[b]** Percentage of total phenotypic variation explained by the QTL, **[c]** QTL detected below the threshold but significant in other shelf life days. Codes were used for all quality traits refer to "trait-day-year".

A distinction is made between major QTL, which account for more than 20% of the explained population variance, and minor QTL which account for less than 20% of the explained population variance (Causse et al., 2002; Kenis et al., 2008; Urrutia et al., 2016). Accordingly, in 2013, three QTL for fruit lightness (L* value) and TSS/TA ratio could be considered as major QTL, whereas 17 of the 39 QTL detected in 2014 were major QTL. In addition, three major QTL in season 2014 accounted for >30% of the phenotypic variance. These QTL were underlying FW-4-14 (LG3A), FW-7-14 (LG3A) and TSS-7-14 (LG5A) with the percentage of 37.5%, 37.8% and 38.2%, respectively. Similar values of the phenotypic variance explained by QTL for agronomical and fruit quality traits in an octoploid strawberry were reported between 9.2% and 30.5% (Zorrilla-

Fontanesi et al., 2011). However, lower values were also reported in another population of an octoploid strawberry in which the phenotypic variation varied from 4.8-17.3% (Lerceteau-Kohler et al., 2012). The latter demonstrated the ability to map minor QTL, i.e. QTL with a small value of phenotypic variance, as a result of using relatively a large population (213 full-sibling).

From the EMR trial (season 2013), eight QTL for five traits were identified on six LGs (Table 4.3). Between 1 and 2 QTL were detected per trait. Among them, 1 QTL (19.3%) was mapped for TSS/TA-1-13, 2 QTL (36.3%) were mapped for L-1-13, 2 QTL (35%) were mapped for L-7-13, 1 QTL (19.9%) was mapped for a-7-13, and 2 QTL (40%) are for TSS/TA-7-13. The total phenotypic variance explained by each individual QTL ranged from 13.4% to 22.9%. While from Reading trial (season 2014), 39 QTL for 19 traits were identified (Table 4.4). Between 1 and five QTL were detected per trait. 1 QTL is for Pel-1-14 (28.9%), Firmness-4-14 (16.4%), L-4-14 (21%), a-4-14 (22.1%), TSS-4-14 (22.8%), TA-4-14 (24.6%), Pel-4-14 (24.7%), Pel-7-14 (22.2%), TA-7-14 (25.4%) and TSS/TA-7-14 (29.3%). 2 QTL are for Cya-1-14 (41.5%) and EA-1-14 (38.5%). 3 QTL are for FW-1-14 (57.2%), FW-4-14 (66.7%), FW-7-14 (66.8%), TSS/TA-4-14 (53.9%) and EA-7-14 (51.3%). 5 QTL are for TSS-7-14 (97.9%) and TSS/TA-1-14 (66.1%). The total phenotypic variance explained by each individual QTL ranged from 7.6% to 38.2%. It is assumed that the difference between the number of QTL identified for season 2013 (8) and for season 2014 (39) is related to

different genotypes used in both years (for more details refer to section 3.3.3). It is worth mentioning that the 20 genotypes that overlapped in both years did not show similar trait data (see section 3.3.5), therefore the environmental effect between the two years was significant and it was not possible to map all the traits/genotype data together but it was necessary to keep them as two distinct datasets.

The number of QTL controlling fruit quality traits varied from 1-5 QTL. TSS and TSS/TA ratio are controlled by the largest number of QTL (5), followed by FW-1-14, FW-4-14, FW-7-14, TSS/TS-4-14, and a-7-14 (3), which may explain the complexity of the biological processes or metabolic pathways underlying these traits (Lerceteau-Kohler et al., 2012), and confirm the quantitative nature of these traits. However, for 12 traits only a single QTL was detected.

For TSS and TSS/TA ratio, five QTL were detected for each as a maximum QTL number per trait, which could suggest that these two above-mentioned traits depend on a large number of factors involving several metabolic pathways for the synthesis, transport, storage and degradation of sucrose, fructose, glucose as well as the organic acids (Etienne et al., 2002; Lerceteau-Kohler et al., 2012; Lobit et al., 2006; Sweetman et al.). Previously, several QTL were also reported in different progeny of an octoploid strawberry for TSS (3-4 QTL) and TA (3-5 QTL) (Lerceteau-Kohler et al., 2012; Zorrilla-Fontanesi et al., 2011).

Over two years, two QTL linked to TSS/TA ratio and fruit lightness (L* value) were common which were therefore assumed to be independent of the environment (Table 4.3 & 4.4). These two QTL were located in LG3A and LG6B for TSS/TA ratio and L* value, with slightly different position for QTL linked with L* value; however, their allelic effects are similar (lmxll). For TSS/TA ratio, these QTL individually accounted for 19.3 and 21.1% of the variation of the total observed variance for season 2013 and 2014, respectively, whereas for L* value, they accounted for 13.4 and 21% of the variation of the total observed variance, for season 2013 and 2014, respectively. This could suggest the presence of the potential allelic forms of the same gene responsible for these particular traits regardless of the different environments. However, other QTL for fruit quality traits were detected only in one year, suggesting a genotypic effect (as only 20% genotypes overlapped in both years) as well as environmental effect (different sites and conditions).

The locations of identified QTL changed in different years, a phenomenon also found in previous studies. For instance, (Wu et al., 2014) detected 32 QTL of fruit quality related traits of pear, however, only 12 of the identified QTL were stable over two successive years. In apple, 26 of 74 identified QTL for the major fruit physiological traits were stable over two harvest years (Kenis et al., 2008). In strawberry, approximately 13 of 33 identified QTL for the agronomical and major fruit quality traits were stable over three harvest years (Zorrilla-Fontanesi et al.,

2011). Most probably, this phenomenon in this current study is related to different genotypes used in both years (20% genotypes overlapped in both years) and to the effect of pre-harvest conditions, including different cultivation sites and conditions (open field in 2013 and glasshouse in 2014). This may suggest the effect of environment (E) and/or G x E interaction on measured traits. Previously, Lerceteau-Kohler et al. (2004) reported similar differences of QTL linked to fruit quality traits in octoploid strawberry between experiments and years.

These potential results of common QTL are tentative (only 20% genotypes overlapped over two years), so further analysis with full-overlapped genotypes over at least two years are encouraged in order to get better evaluation and understanding of the stability of QTL over at least two trials at two different sites. This will enable researchers to compare the stable QTL of the quality traits of strawberry fruit for this particular population with other populations in future, and with a range of elite commercial cultivars, which will facilitate the development of marker-assisted selection approach (MAS), thus moving from phenotype-based towards genotype-based selection. However, prior to applying MAS approach, confirmation steps are required including QTL confirmation, QTL validation and/or fine mapping using high resolution map as shown in (Langridge et al., 2001).

In order to utilize the outcomes of the QTL analysis for MAS approach, preliminary steps need to be undertaken (Collard and Mackill, 2008). First, the

detected QTL have to be validated over different years, growth sites, generations and genetic background in order to evaluate the stability of the QTL. After that, stable QTL need to be refined to a narrower point by saturating that region of the map with additional markers and identify candidate gene/s that map to the refined region. Then, markers that linked to the trait of interest need to be validated through testing these markers in breeding materials. Once tightly linked markers that reliably predict a trait phenotype have been identified, they may be used for MAS.

This study was also carried out to measure the quality traits over two shelf life points in season 2013 and three shelf life points in season 2014 and then the QTL analysis was conducted (Table 4.3 & 4.4). It is interesting to note that QTL for fruit quality traits over shelf life points were detected for 7 out of 11 traits including FW, fruit lightness (L* value), TSS, TA, TSS/TA ratio, ellagic acid, and pelargonidin. However only FW and TSS/TA ratio showed the same QTL localised on the same LG over shelf life points.

For FW, three QTL were detected for all three shelf life points in season 2014 only, which together accounted for 57.2%, 66.7% and 66.8 of the variation for day 1, day 4 and day 7, respectively (Table 4.4). These three QTL are localised to LG3A (83.392 cM), LG6B (52.054 cM), and LG1B (15.703 cM). For TSS/TA ratio, two QTL were detected in the same LG over shelf life points (season 2014), however one of them are located in slightly different location within the LG. The

first QTL was located in LG7A at 0.18 cM for day 4 and day 7, respectively, whereas the second QTL was located in LG6A at 37.024 and 30.635 cM for day 1 and day 4, respectively. Showing such findings suggest the presence of the potential allelic forms of the same gene responsible for these two traits regardless to the shelf life points.

However, the other traits including lightness (L* value), TSS, TA, ellagic acid, and pelargonidin showed different QTL over different shelf life point which could suggest that different places of the chromosomes are regulating the trait depending on the shelf life storage point. In this respect, Kenis et al. (2008) worked on apple and carried out QTL analyses for the same quality traits after storage and/or 10-day shelf life. They revealed that significant differences in the position of QTL after storage as well as QTL co-localisation.

Comparison of the QTL localisation of the current study with previously published study which identified 87 QTL for 19 fruit traits (including fruit development, texture, colour, anthocyanin, sugar and organic acid content), for the F1 progeny derived from the cross of variety “Capitola” x the genotype “CF1116”, based on SSR markers (Lerceteau-Kohler et al., 2012) is possible. Several identified QTL linked to a* value (LG1A), TSS (LG5A), TA (LG2C), and polyphenols content (LG1A, LG2A, and LG6A) were also reported to the same LG by Lerceteau-Kohler et al. (2012). For example, for a* value (a-7-13), QTL was detected on 32.217 cM of LG1A (19.9 %). Previously, QTL linked to

a* value was also detected on LG1A (Lerceteau-Kohler et al., 2012). Although their study did not report the exact position of the QTL in cM, however such comparison may confirm the QTL identification of the current study. Therefore, future work focusing on these LG is required which may help to identify the candidate gene/s controlling those traits that in turn will help to understand the genetic basis of these traits.

4.3.3.2 Epistasis (Gene x Gene interaction)

Epistasis, a phenomenon common to genetics, can be described as a two genes acting together to create a phenotype. It is also known as a "gene x gene interaction" or "locus interaction" which has been recently revealed in QTL studies, making the picture of gene action much more complicated (Jannink and Jansen, 2001; Mao et al., 2011; Mao and Da, 2005; Verhoeven et al., 2010; Wang et al., 1999). Four QTL for three quality traits including TA-7-14, L-1-13, and TSS/TA-1-14 act epistatically, meaning that these loci are genetically dependent on each other (Table 4.5).

Table 4.5. Gene x Gene interaction for four traits.

Tests of Between-Subjects Effects					
Dependent Variable:	**TA_7_14**				
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
AX_89801556_nmh * AX_89874899_nmh	0.022	1	0.022	4.891	0.031
Dependent Variable:	**L_1_13**				
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
AX_89791332_nmh * AX_89877247_nmh	0.008	1	0.008	5.612	0.020
Dependent Variable:	**TSS_TA_1_14**				
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
AX_89844900_ph3 * AX_89894739_nmh	0.047	1	0.047	4.759	0.034
AX_89844900_ph3 * AX_89899527_nmh	0.041	1	0.041	4.175	0.046
Dependent Variable:	**FW_7_14**				
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
AX_89849864_ph3 * AX_89904113_ph3	0.044	1	0.044	3.248	0.078

In maize, it was found that epistasis has a major impact in trait expression (Parvez et al., 2007). From the plant-breeding point of view, epistasis may cause a bias in the estimation of the genetic components and affect the selection processes (Bocianowski, 2013; Parvez et al., 2007). This bias some causes profound consequences such as inaccurate estimates of the expected gain from selection (Eta-Ndu and Openshaw, 1999). However, in some cases, some epistatic gene compensations are favourable (positive epistasis) which can be fixed in the inbreds (Parvez et al., 2007). Thus, this means care should be taken in breeding

programmes to consider further studies to assess the epistatic interactions before considering marker-assisted selection (MAS) for these particular traits.

4.3.3.3 *Co-location and clusters*

Co-localization between detected QTL was assessed for all 24-quality traits (Figure 4.5). Seven LGs showed co-location between QTL including LG1A, LG1B, LG2B, LG3A, LG6A, LG6B, and LG7A. Three QTL mapped for FW-1-14, FW-4-14 and FW-7-14 co-located at LG1B, LG3A and LG6B. Two of the three co-locations (LG1B and LG6B) were with the Hapil allele positive contribution, whereas the third was with the RG allele positive contribution. This suggest that both of the parents contributing to FW trait. Co-localization was also detected at LG7A for QTL linked with TSS/TA-4-14 and TSS/TA-7-14 with the RG allele positive contributing to TSS/TA ratio at day 4 and 7. In addition, co-localization was also detected at LG6A for QTL linked with TSS/TA-1-14 and TSS/TA-4-14 with the Hapil allele positive contributing to TSS/TA ratio at day 1 and 4. Taken together, such findings could suggest that these loci might have gene/s underlying the specific trait irrespective to the storage period.

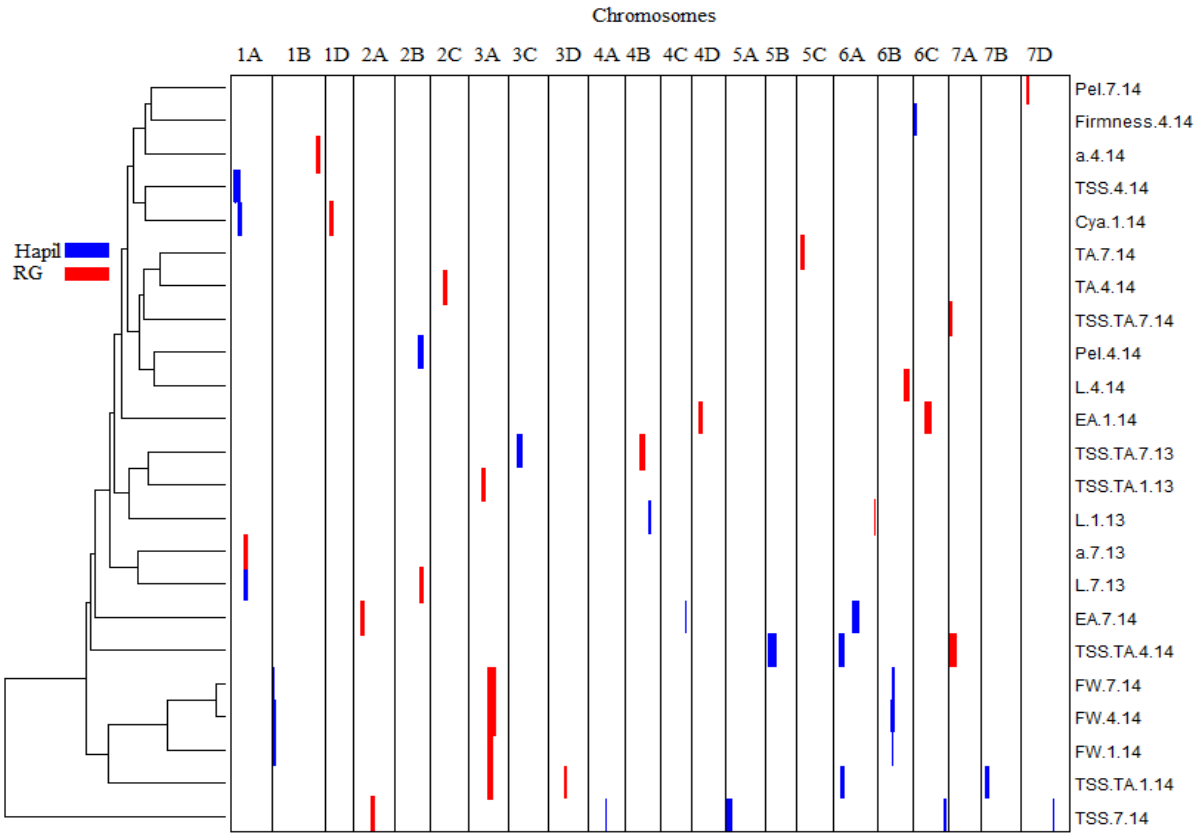


Figure 4.5. A clustered heat map showing the LOD profiles of the measured traits for the RG × H progeny population. Columns indicate the linkage groups (LGs), scaled in centimorgans (cM), ascending from the left to right. Rows indicate individual trait profiles. A colour scale is used to indicate the parental effect. Red colour indicates a positive effect on the trait by the RG, blue colour indicates a positive effect on the trait by the Hapil. The width of a bar indicates the significance interval of the QTL. Hierarchical clustering, shown on the left, reflects the correlation between traits based on the QTL profiles. Codes were used for all quality traits refer to "trait-day-year".

Partial co-localization was also detected for QTL linked with Cya-1-14 and TSS-4-14 at LG1A, both with the Hapil allele positive contributing to higher trait values, suggesting pleiotropic effect at this particular LG. This co-localization may reveal the high correlation of the TSS and cyanidin shown previously in this chapter ($p \leq 0.01$; Pearson's correlation coefficient analysis; section 4.3.2) as two

related traits are more likely to share common QTL (Causse et al., 2002). It is well-known that sugars are the initial precursor of the anthocyanin biosynthesis (Hrazdina et al., 1984; Ruhnan and Forkmann, 1988; Teusch et al., 1987).

Over again at the same LG, co-localization of colour-related QTL was detected for L-7-13 (with Hapil allele positive contribution) and a-7-13 (with RG allele positive contribution). This was also expected because of the high positive correlation of the colour-related parameters ($p \leq 0.01$; Pearson's correlation coefficient analysis; section 4.3.2). Interestingly, QTL for Pel-4-14 (with Hapil allele positive contribution) and L-7-13 (with RG allele positive contribution) were co-located at LG2B, which is the only co-location identified for different years/conditions, suggesting antagonistic pleiotropic effect. This was in agreement with the negative correlations found between them ($p \leq 0.01$; Pearson's correlation coefficient analysis; section 4.3.2) which may explain the commonalities in the genetic regulation between anthocyanin content and the redness of strawberry fruit.

According to the correlation data observed earlier in this chapter, only pelargonidin negatively significantly correlated to colour parameters (L*, a* and b* values) for both years which means with the L* value decrease (fruit become darker) with an increase of pelargonidin content. Co-location of anthocyanin and colour was also reported in strawberry (Zorrilla-Fontanesi et al., 2011) and raspberry (McCallum et al., 2010). The candidate gene approach has been

previously used and an association between R2R3-MYB transcription factors and QTL controlling fruit colour and anthocyanins have been reported in strawberry, apple, sweet cherry and raspberry (Chagné et al., 2007; McCallum et al., 2010; Sooriyapathirana et al., 2010; Zorrilla-Fontanesi et al., 2011).

Our data showed that co-locations of QTL for different quality traits were uncommon suggesting that different places of the genome control different traits. However, several clusters of QTL were identified mainly on LG1, LG3, and LG6 (9, 7, and 11 QTL, respectively) (Table 4.6). For LG1, nine QTL were identified (four QTL on LG1A, four QTL on LG1B, and one QTL on LG1D) for several traits including FW, L* value, a* value, TSS, and cyanidin. For LG3, nine QTL were identified (five QTL on LG3A, one QTL on LG3C, and one QTL on 3D) for two traits, those are FW and TSS/TA ratio. Finally, for LG6, eleven QTL were identified (three QTL on LG6A, five QTL on LG6B, and three QTL on LG6C) for several traits including FW, L* value, TSS/TA ratio, ellagic acid, and firmness. Previous QTL studies in tomato (Fulton et al., 1997) and maize (Edwards et al., 1987) noted that particular regions of the genome influenced several traits.

Table 4.6. Distribution of QTL locations on the 28 linkage groups (LG).

Linkage groups	No. of QTL				Total
	a	b	c	d	
1	4	4	0	1	9
2	2	2	1	0	5
3	5	0	1	1	7
4	1	3	1	1	6
5	1	1	1	0	3
6	3	5	3	0	11
7	2	1	0	2	5

In strawberry, similar clusters of QTL linked to fruit quality, including fruit development, texture, colour, anthocyanin, sugar and organic acid content, were located on homoeology groups HG3 (LG3A, LG3B, LG3C, and LG3D) and HG6 (LG6A, LG6B, LG6C, and LG6D) based on SSR markers (Lerceteau-Kohler et al., 2012). These results of co-location and/or clusters perhaps reveal a pleiotropic effects, different genes with close linkage (Chen et al., 2015; Lerceteau-Kohler et al., 2012), or the segregating of common QTL regulate two traits as a result of a causal relationships among them or of related metabolism (Causse et al., 2002).

QTL clusters in this experiment reflect the level of correlations noted previously. Therefore, these LGs with clustered QTL may have the potential to develop strawberry fruit quality. Thus, further study focusing on these LGs (LG3 and LG6) within the confidence intervals of identified QTL may help to assess the mechanism for controlling traits of interest.

4.3.4 Refine QTL position

An attempt was made to benefit from the available saturated SNP linkage map (3933 SNPs). Six different density linkage maps were created, with different number of markers, from the original saturated map and tested for QTL analysis in order to refine the QTL position through the saturation of the regions under the significant QTL with as many markers as possible (Table 4.7). Accordingly, the best map (in term of powerful and no computational problems with mapQTL which cannot function if too many markers are presented to it) is the map with 523 SNP (5 cM interval). Then, the map was refined by using a step-wise-approach (map with 238 SNP, 10 cM interval and 1 cM on the positions of high significant QTL) on seven traits only including L-1-13, FW-1-14, TSS/TA-1-14, FW-4-14, TSS/TA-4-14, FW-7-14, and TSS-7-14 (those have QTL higher than LOD detected by permutation test).

Table 4.7. Details of the six different density linkage maps.

Analysis no.	No. of markers	Interval (cM)	Saturation	no. of LGs	Result
1	523	5	No Saturation	28	√
2	270	10	No Saturation	28	√
3	209	10	No Saturation	22	√
4	679	5	1 cM on the positions of QTL	28	x
5	565	5	1 cM on the positions of QTL	22	x
6	328	10	1 cM on the positions of QTL	28	√

√ means mapQTL run successfully. **X** means mapQTL did not run successfully.

Seven traits were used for the refinement of QTL positions those have significant QTL (QTL higher than LOD obtained by the permutation test, LOD > 5). The regions characterized by these significant QTL were saturated based on the step-wise approach (map with 328 SNP, 10 cM interval and 1 cM on the positions of high significant QTL). Nine Major QTL were in question to refine positions and the results of the refinement are summarized on Table 4.8. Six QTL (out of nine major QTL) remained the same after adding 1 cM intervals on the position of QTL, which indicates the precision of the 5 cM map. Furthermore, one QTL for TSS-7-14 has slightly shifted within the same LG which may could suggest that the marker at the position of 20.052 cM is the nearest, however the LOD decreased from 13 (for the 5 cM map) to 7.18 (for the saturated map). Finally, two QTL for TSS/TS-1-14 and L-1-13 became non-significant and instead two new QTL became significant. Adding/removing markers allow for shifts, some new QTL and might lose QTL. This can be of two factors; the interaction and the epistasis between loci.

Table 4.8. QTL detected by two different density maps.

Trait	Map with 523 SNPs (5 cM interval)			Map with 328 SNPs (10 cM interval)		
	LOD	LG	Position (cM)	LOD	LG	Position (cM)
FW-1-14	**5.21**	**LG3A**	**83.392**	**5.21**	**LG3A**	**83.392**
TSS/TA-1-14	5.4	LG3A	89.707	7.35	LG6C	12.103
FW-4-14	**7.97**	**LG3A**	**83.392**	**7.97**	**LG3A**	**83.392**
TSS/TA-4-14	**5.11**	**LG7A**	**0.18**	**5.26**	**LG7A**	**0.18**
FW-7-14	**7.97**	**LG3A**	**83.392**	**6.49**	**LG3A**	**83.392**
TSS-7-14	13	LG5A	24.383	7.18	LG5A	20.052
	10.3	**LG7D**	**45.015**	**4.53**	**LG7D**	**45.015**
	7.13	**LG6C**	**95.604**	**3.84**	**LG6C**	**95.604**
L-1-13	5.26	LG4B	70.412	3.36	LG4D	51.981

Those QTL identified the same in two maps are marked in bold; the QTL shifted within the same LG in two maps is highlighted.

4.4 **Summary**

Mapping QTL in octoploid strawberry is challenging, because of its ploidy and genome complexity. In this chapter, the aim was to assess the segregation of the population for the traits of interest, the correlation between these traits, and to identify the candidate QTL linked to the traits of interest over different shelf life days using a SNP-based linkage map. Over two sequential seasons, 47 QTL were mapped for 51 quality traits and several of them collocated suggesting possible pleiotropic effects. 22 of the 47 identified QTL were ‘major’ QTL, accounting for over 20% of the observed population variance of the trait. Beside the fact that only 20% of genotypes are overlapped between the trials, few common QTL linked with the quantitative traits were detected highlighting a strong environmental

effect on the genetic architecture of these traits, validated by significant G x E interactions.

All these results represent a good starting point for further work, as they indicate the most likely candidate regions influencing polyphenols production and other quality traits. However, it is still necessary to confirm the stability of the identified QTL resulting from the current study in other mapping population of an octoploid strawberry, at different environment, and over several years at least two before they are considered in breeding programmes for MAS.

As a final remark, it is worth mentioning that to date, QTL studies of strawberry quality traits have focused on traits measured at harvest, while the majority of fruit reaches the consumer only after a period of up to several days in cold storage and post-harvest storage. Therefore, this study was also carried out to measure the quality traits over different shelf life points and then the QTL analysis was conducted. In the present study, QTL for fruit quality traits over different shelf life points were detected for 7 out of 11 traits (FW, fruit lightness (L* value), TSS, TA, TSS/TA ratio, ellagic acid, and pelargonidin), however only FW and TSS/TA ratio showed the same QTL localised on the same LG over shelf life points. This could suggest that the presence of the potential allelic forms of the same gene responsible for those two traits regardless to the shelf life points, whilst the other traits showed different QTL over shelf life points suggesting that different genes are regulating a single quality trait depending on the shelf life storage.

Chapter 5 : Sensory analysis of nine genotypes of an F1 strawberry (*Fragaria x ananassa*) and comparison with instrumental analysis

5.1 Introduction

Cultivated strawberry (*Fragaria x ananassa*) is highly considered for their health benefits and unique flavour. It is one of the most attractive fruits, which is consumed as fresh, conserved, or as manufactured products. Volatile (aroma compounds) and non-volatile (sugar and organic acid) compounds are believed to be responsible for strawberry flavour.

More than 350 volatile compounds have been identified in strawberries, however their relative contribution to aroma depends on their concentrations and on their odour detection threshold (Azodanlou et al., 2003; Bood and Zabetakis, 2002; El Hadi et al., 2013; Forney et al., 2000; Hakala et al., 2002; Pelayo et al., 2003; Schwieterman et al., 2014). These volatiles were classified in five classes of chemicals as major flavour contributors in fruit: esters, alcohols, aldehydes, ketones and terpenoids (Kader, 1997), with esters and furanones being reported as the main strawberry flavour compounds (Song and Forney, 2008; Zabetakis and Holden, 1997). Methyl butanoate, ethyl butanoate, methyl hexanoate, cis-3-hexenyl acetate, and linalool were reported to be the major volatile compounds in strawberry (Azodanlou et al., 2003). They have long been recognised as playing multiple roles in plants including attracting insects for seed dispersion and

pollination, revealing that fruit are ripe and ready for seed dispersal, and modulating systemic acquired resistance to pests and diseases as part of the plant defence system (Ceuppens et al., 2015; Rowan, 2011).

Strawberry has one of the most complicated flavours among flowering plants (Zabetakis and Holden, 1997). This is due to the large number of volatile (aroma) and non-volatile (sugar and organic acid) compounds linked to strawberry flavour. Previous studies have investigated the relationship between sensory attributes and instrumental analysis in strawberry (Gunness et al., 2009; Jouki and Dadashpour, 2012; Jouquand et al., 2008; Pelayo et al., 2003; Resende et al., 2008; Schwieterman et al., 2014; Ulrich et al., 2006) and melon (Lignou et al., 2014). In strawberry fruits, instrumental analysis such as TSS/TA ratio and pH were a good predictors for sensory perception such as sweetness, sourness and flavour intensity of the fruit (Gunness et al., 2009). In melons, the sensory analysis was linked well with instrumental data (Lignou et al., 2014). Such findings indicate that the instrumental analysis is a good guide for sensory perception.

The aim of this experiment was to evaluate the flavour profiles of seven genotypes of an F1 strawberry (*Fragaria x ananassa* Duch.), derived from the cross of Redgauntlet x Hapil (RGxH), plus the parental lines, at two shelf life points (day 1 and day 5) of storage at a commercially relevant temperature of 4 °C. A second aim was to examine correlations between sensory attributes, volatiles and physicochemical data. As the target of the experiment was flavour analysis, the

selection of the nine genotypes from within the whole RGxH population was based on sugar and acid content (TSS, TA, and TSS/TA ratio). Fruits had different TSS and TA were selected, so that the taste is likely to be distinctive enough to show differences in sensory attributes. Ten trained sensory panellists rated strawberry puree samples stored at 4 °C (day 1 and day 5), while all physicochemical traits including fresh weight (FW), firmness, colour (L*, a*, and b* values), total soluble solids (TSS), titratable acidity (TA), volatile compounds and phenolic compounds were measured according to the detailed procedures in Chapter 2.

5.2 Materials and methods

The materials and methods used for the experiments in this chapter are described in detail in Chapter 2.

5.3 Results and discussion

Physicochemical traits of strawberry fruits are a means of applying quantitative measurements to represent fruit quality characteristics as perceived by consumers. These traits, include physical traits (FW, firmness, and colour) and chemical (TSS, TA, TSS/TA ratio and phenolic content), are important for consumers and therefore may directly influence producers, suppliers and commercial retailers. The current experiment focussed on nine genotypes (Table 5.1) to assess the flavour profile. This assessment includes the range of physicochemical traits described above, non-volatile compounds, and volatile compounds produced by

different genotypes/shelf life points, and then to examine if there are significant correlations between sensory and instrumental data.

5.3.1 Physicochemical traits

The effect of genotype (G) and storage (E) on the physical and chemical constituents of RG x H population was investigated over a two-year harvesting seasons (year 2013 and year 2014; day 1, 4 and 7) in Chapter 3. However, for the purpose of flavour profile, the assessment of the physical and chemical traits was conducted at two post-harvest days (day 1 and 5), as shown in Table 5.1. Generally, FW, TSS, TA, TSS\TA ratio, ellagic acid and pelargonidin were found to be statistically non-significant with storage time (Table 5.1).

Table 5.1. Mean values for physicochemical traits of the nine genotypes of RGxH progeny at two different shelf life days. TSS (ºBRIX), TA (%), FW (g), and firmness (N).

Trait	Day	Mean values									*P*[a]	*P*[b]		
		RG	Hapil	RG010	RG086	RG098	RG100	RG126	RG164	RG169		Genotype	Day	Interaction
FW	1	7.6[b]	18.7[a]	7.8[b]	9.5[b]	8.3[b]	8.6[b]	7[b]	9.6[b]	8.6[b]	< 0.0001	< 0.0001	NS	NS
	5	7.1[b]	17.8[a]	7.1[b]	8.8[b]	7.7[b]	7.6[b]	6.4[b]	8.8[b]	8.1[b]				
Firmness	1	8.9[abcd]	10.5[ab]	**10.7[ab]**	11.2[ab]	10.3[abc]	10.4[abc]	9.2[abcd]	9.3[abcd]	**12[a]**	< 0.0001	NS	< 0.0001	NS
	5	6[bcd]	5.9[bcd]	**4.2[d]**	7.8[abcd]	6.5[abcd]	6.1[bcd]	4.7[cd]	4.4[d]	**5.6[bcd]**				
TSS	1	5.6[b]	8.1[ab]	9[a]	8.4[ab]	9.9[a]	9.3[a]	8[ab]	8.3[ab]	9.7[a]	0.003	0.0002	NS	NS
	5	6.9[ab]	9[a]	9[a]	8.8[ab]	9.5[a]	8.4[ab]	9.6[a]	8.7[ab]	9.9[a]				
TA	1	0.8[ab]	0.8[ab]	0.9[ab]	1[a]	0.9[ab]	0.9[ab]	0.9[ab]	0.8[ab]	0.9[ab]	0.007	0.001	NS	NS
	5	0.7[a]	0.8[ab]	0.7[b]	0.9[ab]	1[ab]	0.9[ab]	0.8[ab]	0.9[ab]	1[ab]				
TSS/TA %	1	7.1[b]	10.4[ab]	10.7[ab]	8.1[ab]	11.1[ab]	10.6[ab]	9[ab]	10.2[ab]	10.7[ab]	0.011	0.008	NS	NS
	5	9.4[ab]	11.7[a]	12.3[a]	9.5[ab]	9.5[ab]	9.5[ab]	11.3[a]	9.8[ab]	9.7[ab]				
L* value	1	36	33.7	32.4	33.2	37.1	36.3	36.7	34.4	33.1	NS	0.05	0.005	NS
	5	30.7	30	31.1	30.6	35.7	34.8	34.9	31.6	31.2				
a* value	1	30.6[a]	24.3[abcde]	26[abcde]	27[abcde]	22.2[cde]	26.7[abcde]	30[ab]	27.7[abcd]	**29.4[abc]**	< 0.0001	0.001	< 0.0001	NS

Trait	Day	Mean values									P^a	P^b		
		RG	Hapil	RG010	RG086	RG098	RG100	RG126	RG164	RG169		Genotype	Day	Interaction
b* value	5	25^{abcde}	22.9^{abcde}	22.9^{abcde}	25.2^{abcde}	19.6^{e}	22.7^{bcde}	26.2^{abcde}	21.7^{cde}	**21.5^{de}**	0.0002	0.003	< 0.0001	NS
	1	20.7^{ab}	16.3^{abc}	16.8^{abc}	19.5^{abc}	11.7^{c}	19.5^{abc}	22.1^{a}	19.4^{abc}	17.5^{abc}				
	5	15.6^{abc}	13.9^{abc}	13.5^{abc}	16.9^{abc}	11.1^{c}	13^{bc}	14.7^{abc}	13.7^{abc}	11.8^{c}				

a Probability, as obtained from one-way ANOVA, that there is a difference between means; NS: no significant difference between means ($P \leq 0.05$). **b** Probability, as obtained from two-way ANOVA, that there is a difference between means; NS: no significant difference between means ($P \leq 0.05$). Superscript letters for each trait indicate differing levels of significance for each respective genotype and/or day (ANOVA Tukey's HSD test; ($P \leq 0.05$)). Those scores significantly different between the two shelf life days are marked in bold.

5.3.1.1 FW

FW decreased with storage time for all seven F1 genotypes, plus the parents, although it was not statistically significant. Such a decrease was expected, as strawberries are very susceptible to water loss that leads to several consequences, one of which was weight reduction as it was described earlier in Chapter 3 (for more results refer to section 3.3.5.7).

5.3.1.2 Firmness

A significant decrease was found in firmness over shelf life for all seven F1 genotypes plus the parents ($p<0.0001$) (Table 5.1). The decrease was in agreement with the reported results in Chapter 3 (section 3.3.5.6) as well as with the fact that strawberry softening increases with ripening and storage (Ali et al., 2011; Nunes et al., 1995). The decrease in the firmness is due to the degradation of the middle lamella of the cell wall, which is regulated by *polygalactunase* enzyme (FaPG1), (Ali et al., 2011; Almenar, E. et al., 2007; Figueroa et al., 2010; Jouki and Dadashpour, 2012; Molina-Hidalgo et al., 2013; Rees et al., 2012; Trainotti et al., 1999; Vicente et al., 2005) and causing a loss of fruit turgidity (Valenzuela et al., 2015).

5.3.1.3 TSS, TA and their ratio

TSS during post-harvest storage at 4 ºC showed an increase in both parental lines however this increase was insignificant (Table 5.1). Among the seven offspring lines, divergent results were obtained where five of them, including RG010,

RG086, RG126, RG164, and RG169, showed an increase during the post-harvest storage, while RG098 and RG100 showed a decrease. Such an increase of the TSS during storage could be attributed to the new soluble sugar biosynthesis which could take place again using a carbon supply which results from cell wall disassembly as a precursor (Cordenunsi et al., 2005; Jouki and Dadashpour, 2012; Schwieterman et al., 2014), however the decrease suggest the hydrolysis of sucrose during storage, as strawberry fruit has very small amount of starch (Mishra and Kar, 2014; Pelayo et al., 2003). Similar trends of divergence between the offspring lines were obtained previously (for more results refer to section 3.3.5.1). Such divergence might be attributed to a genetic variability within the offspring lines due to the divergence of these parameters between the parents that were used to generate the mapping population.

Beside the differences in the TSS content between the genotypes, the minimum TSS result recorded was 5.6 (RG-day 1) and the maximum was 9.9 (RG098-day1 & RG169-day 5). The recommended range of the total soluble solids in strawberries used in commercial practice is 7-12 ºBRIX, depending on the genotype (Ayala-Zavala et al., 2004). In comparison with the reported TSS data stored at 4 ºC for 7 days, the minimum TSS result recorded for season 2014 was 5 and the maximum was 11.2 for day 1. Such variability might be attributed to year-to-year influence (Jouquand et al., 2008), the different number of lines have

been used (76 lines for season 2014 and nine lines for season 2015), and to the different length of storage (7 days for season 2014 and 5 days for season 2015).

Trends of TA content were found to be different between genotypes as well, however none of them were significant. The parental lines showed a decrease as well as RG010, RG086, RG126. However, three offspring lines showed an increase during post-harvest storage (RG098, RG164, and RG169), while RG100 showed no difference between the two shelf life days (day 1 and 5). Similar trends of divergence between the offspring lines were obtained previously (for more results refer to section 3.3.5.2). Accordingly, trends of TSS/TA ratio content were found to be different between genotypes. TSS/TA ratio during post-harvest storage at 4 ºC showed an increase in both parental lines (Table 5.1), while three offspring lines including RG010, RG086, and RG126 increased during the post-harvest storage (as their TSS content increased during storage while TA content decreased). On the other hand, the other four lines (RG098, RG100, RG164, and RG169) decreased (as their TSS content decreased during storage while TA content increased).

5.3.1.4 Colour parameters (L, a* and b*)*

Changes in colour parameters (L*, a*, and b* values) during shelf life were monitored (Table 5.1). All nine genotypes showed a decrease for all colour parameters with increasing post-harvest storage. Such low values of L* (more darkness), a* (more red) and b* (more blue) at day 5 indicate overall darker fruit

colour. This is believed to be the result of the accumulation of anthocyanins, which is known as a major pigment in strawberry fruit (Hancock, 1999; Kosar et al., 2004), and decrease of chlorophyll synthesis during ripening process (Cited by Civello and Martínez, 1997), as the majority of the lines showed an increase of anthocyanins (pelargonidin and cyanidin) during the post-harvest storage of 5 days. The decrease was in agreement with the reported results of colour parameters during the shelf life storage in Chapter 3 (section 3.3.5.4). More evidence about the effect of genotype (G) and storage (E) on the physicochemical traits of RGxH population was investigated in details over two-year harvesting seasons (year 2013 and year 2014) in Chapter 3 (sections 3.3.4 and 3.3.5).

5.3.1.5 Non-volatile compounds

Changes in three phenolic compounds (ellagic acid, pelargonidin and cyanidin) were evaluated during the shelf life (Table 5.2). Ellagic acid is the major phenolic acid in strawberry, while pelargonidin and cyanidin are the major anthocyanins in strawberry. No significant differences were found for these compounds during shelf life storage with the exception of cyanidin (Table 5.2; one-way ANOVA). It has been previously found that pelargonidin and cyanidin were significantly increased during shelf life in season 2014 ($p<0.001$) (for more details see Table 3.3 & 3.4). Thus, the increase of anthocyanins was in agreement with the reported results of season 2014 in Chapter 3 (section 3.3.5.5), however this increase was significant for cyanidin only. The reason behind this could be attributed to the

different number of lines have been used (76 lines for season 2014 and nine lines for season 2015) as well as to the difference in the length of the postharvest storage between the current experiment (up to 5 days) and the previous one (up to 7 days; Chapter 3).

Table 5.2. Mean values for non-volatile compounds of the nine genotypes of RGxH progeny at two different shelf life days. Ellagic acid, pelargonidin and cyanidin content (mmol/g FW).

Trait	Day	Mean values									P^a	P^b		
		RG	Hapil	RG010	RG086	RG098	RG100	RG126	RG164	RG169		Genotype	Day	Interaction
Ellagic acid	1	5	3.5	5	5.4	4.7	7	6	6.4	5.8	NS	NS	NS	NS
	5	5.4	4.3	5.7	5.6	5.6	5.1	7.9	4.7	7				
Pelargonidin	1	2.9	2.9	3.3	2.5	4	2.5	2.1	3.2	4.2	NS	0.004	NS	NS
	5	2.8	3.4	3.5	3.1	4.6	2.4	2.7	3	4.7				
Cyanidin	1	0.7	0.4	0.5	0.5	0.3	0.4	0.6	0.5	0.6	NS	0.041	0.022	NS
	5	0.7	0.5	0.6	0.6	0.5	0.5	0.7	0.5	0.8				

[a] Probability, as obtained from one-way ANOVA, that there is a difference between means; NS: no significant difference between means ($P \leq 0.05$). **[b]** Probability, as obtained from two-way ANOVA, that there is a difference between means; NS: no significant difference between means ($P \leq 0.05$).

Phenolic acids are known to act as antioxidants and herbivory defence molecules in plants exposed to any kind of stress (Mithöfer and Boland, 2012; Skłodowska et al., 2011; Treutter, 2006). Strawberries contain both ellagic acid and its glucoside. Ellagic acid content of the nine genotypes of strawberry fruits ranged from 3.5 to 7 mmol/g FW. This is in accordance with the results obtained in the previous year (season 2014 in Reading) when the ellagic acid content for the whole population ranged from 1.4 to 6.59 mmol/g FW (for more results refer to section 3.3.3.1). Among the parental lines, RG had higher amounts of ellagic acid than fruits of the other parent "Hapil", which is again in alignment with the previous results reported in Chapter 3 (section 3.3.3.1). Anthocyanin content is important for the attractiveness and quality of strawberry. Pelargonidin was the main pigment found (Table 5.2). Pelargonidin content of the nine genotypes of strawberry fruits ranged from 2.1 to 7.9 mmol/g FW, while cyanidin content ranged from 0.3 to 0.8 mmol/g FW.

Five out of the seven F1 offspring showed an increase of all phenolic compounds (ellagic acid, pelargonidin and cyanidin) at day 5, however this increase was non-significant. These lines are RG010, RG086, RG098, RG126 and RG169. Whereas, the parental line 'RG' increased for ellagic acid, decreased for pelargonidin and being stable for cyanidin. The parental line 'Hapil' increased for ellagic acid and pelargonidin, but decreased for cyanidin. Such divergent tendencies were also observed among the F1 progeny where in season 2013; 61 % increased and 39 %

decreased in ellagic acid content, while in season 2014, 33 % increased and 67 % decreased in concentration of the same compound (section 3.3.5.5). A possible explanation for the increase of anthocyanins during storage is that the synthesis process of anthocyanins may take place during storage as found previously by Cordenunsi et al. (2005). It is well known that the content of phenolic acid, flavonols, and anthocyanins are increased with increasing storage, but this increase was significantly influenced by temperature as the respiratory metabolism rate increased with increasing temperature (Aaby et al., 2012; Cordenunsi et al., 2005; Kalt et al., 1999; Wang and Zheng, 2001).

5.3.1.6 Volatile compounds

The volatile aroma profile of fruits certainly represents an important factor in consumer perception of sensory properties. In total, 61 compounds were identified in the headspace of the nine genotypes of the strawberry population at two different shelf life points (day 1 and day 5) (Table 5.3). The most abundant compounds in terms of the number of detected compounds and quantities on both days were esters, followed by aldehydes and terpene derivatives (Table 5.3). These included 31 esters (acetates and non-acetate esters), 8 terpene derivatives, 4 alcohols, 9 aldehydes, 2 furanones, 5 carboxylic acids and 2 ketones, compounds previously reported in strawberry (Bood and Zabetakis, 2002; Du et al., 2011a; Forney et al., 2000; Hakala et al., 2002; Jouquand et al., 2008; Ménager

et al., 2004; Pelayo-Zaldivar et al., 2007; Song and Forney, 2008; Zabetakis and Holden, 1997; Zhang et al., 2009).

Table 5.3. List of identified volatile compounds by SPME method in strawberry fruits (*Fragaria x ananassa*) at two different shelf life days (4 °C).

Code	Compound	LRI[a]	ID[b]	Refrence	Code	Compound	LRI[a]	ID[b]	Refrence
Ester					***Terpenes***				
e1	methyl acetate	<600	A		t3	eucalyptol	1039	A	
e2	ethyl acetate	613	A		t4	beta-ocimene	1049	B	Reverchon et al. (1997)
e3	methyl propanoate	629	A		t5	alpha-terpinolene	1094	A	
e4	isopropyl acetate	660	A		t6	linalool	1100	A	
e5	ethyl propanoate	712	A		t7	alpha-terpineol	1199	A	
e6	methyl butanoate	723	A		t8	cis-geraniol	1255	A	
e7	ethyl 2-methylpropanoate	759	A		***Alcohol***				
e8	2-methylpropyl acetate	774	A		a1	1-hexanol	868	A	
e9	methyl 2-methylbutanoate	778	A		a2	benzaldehyde	964	B	Goodner (2008)
e10	ethyl butanoate	801	A		a3	1-octanol	1069	A	
e11	butyl acetate	842	A		a4	(E)-2-hexen-1-ol	866	A	
e12	methyl pentanoate	851	A		***aldehydes***				
e13	isopropyl butanoate	876	A		ald1	3-methylbutanal	651	A	
e14	ethyl 2-methylbutanoate	879	A		ald2	pentanal	697	A	
e15	3-methylbutyl acetate	913	A		ald3	hexanal	800	A	
e16	2-methylbutyl acetate	924	A		ald4	heptanal	901	A	
e17	pentyl acetate	939	A		ald5	nonanal	1104	A	

Code	Compound	LRI[a]	ID[b]	Refrence	Code	Compound	LRI[a]	ID[b]	Refrence
e18	methyl hexanoate	955	A		ald6	(E)-2-hexenal	855	A	
e19	4-methyl-2-heptanone	998	C		ald7	(E,E)-2,4-hexadienal	912	A	
e20	2-methylpropyl butanoate	1011	A		ald8	(E)-2-heptenal	957	A	
e21	ethyl hexanoate	1109	A		ald9	(Z)-2-decenal	1263	A	
e22	hexyl acetate	1148	A		***Furanones***				
e23	heptyl acetate	1167	A		f1	furaneol	1053	A	
e24	2-methylpropyl hexanoate	1188	A		f2	mesifuran	1061	B	Pino et al. (2005)
e25	benzyl acetate	1193	A		**Carboxylic acids**				
e26	hexyl butanoate	1207	A		c1	acetic acid	<600	A	
e27	ethyl octanoate	1204	A		c2	2-methylpropanoic acid	753	A	
e28	octyl acetate	1460	A		c3	butanoic acid	783	B	Young and Baumeister (1999)
e29	methyl salicylate	1005	A		c4	hexanoic acid	975	B	Kondjoyan; and Berdagué (1996)
e30	(Z)-3-hexen-1-ol acetate	842	A		c5	octanoic acid	1160	B	Alañón et al. (2009)
e31	(E)-2-hexen-1-ol acetate	851	A		***Ketones***				
Terpenes					k1	3-octanone	986	B	Sparkman (2005)
t1	beta-pinene	992	A		k2	acetophenone	1072	A	
t2	d-limonene	1035	A						

[a] linear retention index (LRI) on DB-5 column. [b] A: mass spectrum and LRI agree with those of authentic compound; B: mass spectrum agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature; C: mass spectrum agrees with reference spectrum in the NIST/EPA/NIH mass spectra database.

Strawberry aroma is generally linked to the complex mixture of different volatiles including esters, furanones, aldehydes, alcohols, terpenes, lactones, and sulphur compounds (Dirinck et al., 1981; Du et al., 2011a, 2011b; Pyysalo et al., 1979). Quantitative differences were observed between the nine genotypes and the two shelf life points (ANOVA with post hoc Tukey's HSD test; Table 5.4). The most abundant volatile compounds in terms of concentration on day 1 were esters (74.4 %), followed by aldehydes (12.6 %), terpenes (4.2 %), furanones (3.6 %), carboxylic acids (3.3 %), alcohols (1.6 %), and then ketones (0.1 %) (Figure 5.1.a). However, the most abundant volatile compounds on day 5 were also esters (68.2 %), followed by aldehydes (16 %), carboxylic acids (5 %), terpenes (4.5 %), furanones (4.1 %), alcohols (2.1 %) and then ketones (0.1 %) (Figure 5.1.b). This is in agreement with previous study in ripe '*Camarosa'* strawberry where esters were the most abundant volatile compounds (Pelayo-Zaldivar et al., 2007).

Esters, terpenes, aldehyde and furanones were reported as the major aroma compounds in strawberry (Jouquand et al., 2008). Esters and aldehydes are known to contribute to the fresh and fruity strawberry flavour. The intense aroma of the wild strawberry species, which is known for its nice flavour, was associated with the high content of esters and terpenes (Ulrich et al., 2007). Esters and furanones were the most abundant volatile compounds in ripe '*Camarosa'* strawberry.

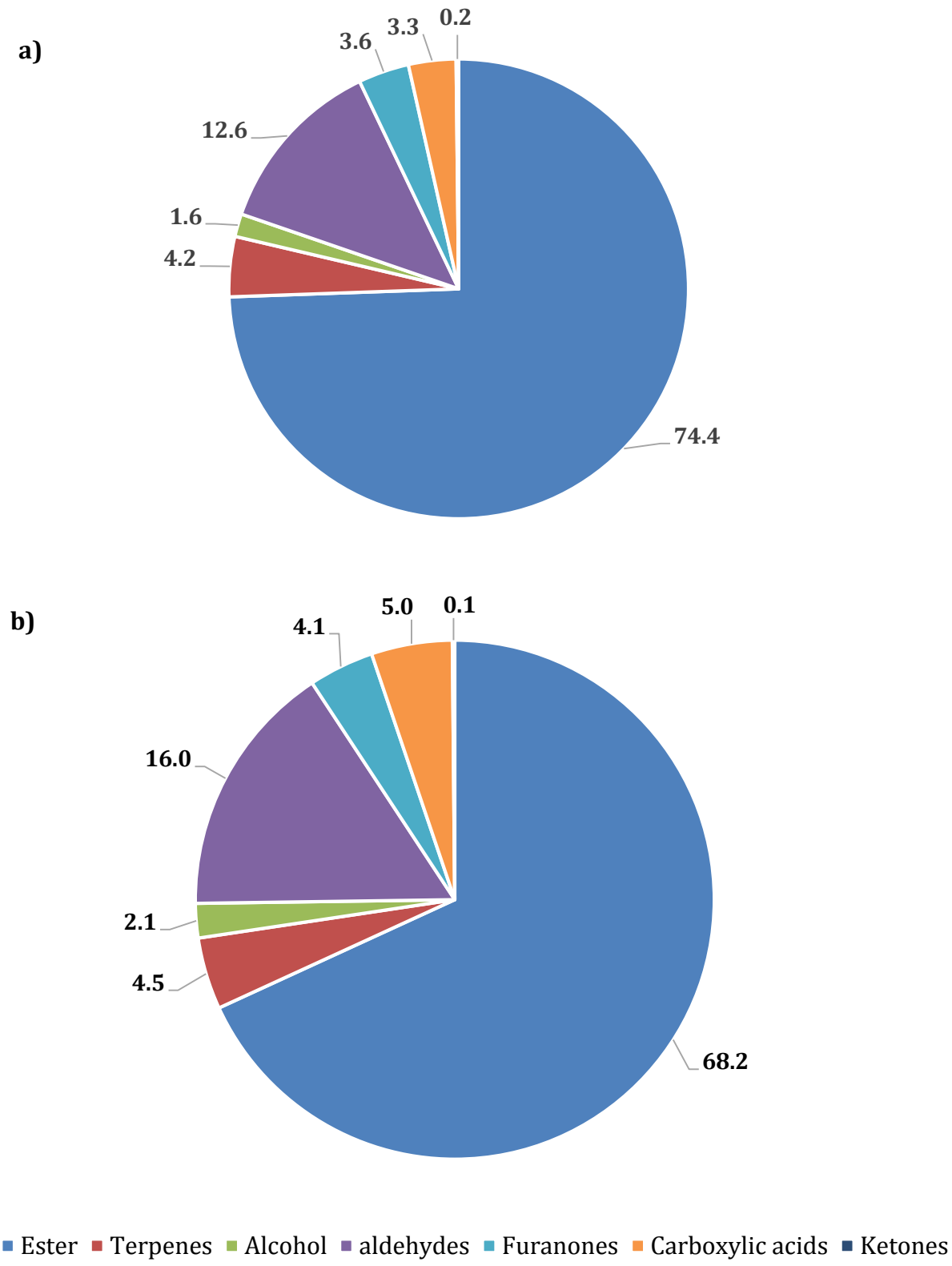


Figure 5.1. The distribution of volatile compounds in the nine genotypes of the strawberry population at two different shelf life points; (a) day 1 and (b) day 5. Values are the percentage of the combined quantities of the groups of compounds.

The parental lines “RG” and “Hapil” displayed similar relative content for several volatile compounds, such as the majority of terpenes, alcohols, ketones and furanones, but line “RG” displayed higher relative concentration of esters and carboxylic acids, while line “Hapil” displayed higher relative concentration of aldehydes. These differences were significant in term of genotype for all identified volatile compounds, with the exception of compounds methyl propanoate (e3), ethyl butanoate (e10), methyl pentanoate (e12), ethyl octanoate (e27), methyl salicylate (e29), eucalyptol (t3), benzaldehyde (a2), 1-octanol (a3), octanoic acid (c5), and acetophenone (k2) (Table 5.4). The F1 offspring lines displayed different concentrations of the compounds, which could suggest the effect of genotype. Previously, Zorrilla-Fontanesi et al. (2012b) investigated the volatile compounds in a strawberry mapping population derived from genotype ‘1392’ and ‘232’ and found that the parental lines ‘232’ and ‘1392’ displayed similar relative content for several volatile compounds, including alcohols and esters, but line ‘1392’ (selected for good flavour) displayed higher concentration of aldehydes, ketones, furans and terpenes. However, these volatiles were reported to be influenced by the storage time and temperature (Ayala-Zavala et al., 2004).

Table 5.4. Approximate quantities of volatile compounds identified in the headspace of SPME extracts of strawberry of the nine genotypes of RGxH progeny measured at two different shelf life days (**n = 3)**.

Code	Day	Approximate quantity[a]									P[p]	P[c]		
		RG	Hapil	RG010	RG086	RG098	RG100	RG126	RG164	RG169		Genotype	Day	Interaction
Ester														
e1	1	203.8	375.1	340.6	499.6	323.1	184.6	143.4	269.7	473.3	0.043	0.002	NS	NS
	5	144.5	425.7	249.5	571.5	238.1	235.6	92.0	247.8	425.7				
e2	1	64.8^{b}	127.2^{ab}	428.5^{ab}	613.1^{ab}	280.3^{ab}	1198.5^{ab}	184.8^{ab}	537.0^{ab}	2191.0^{ab}	0.007	0.007	NS	0.041
	5	40.3^{b}	426.2^{ab}	240.9^{ab}	441.4^{ab}	108.4^{ab}	174.8^{ab}	130.5^{ab}	2308.4^{a}	711.5^{ab}				
e3	1	20.4	11.3	19.9	19.3	31.1	18.8	8.9	49.4	23.5	NS	NS	NS	NS
	5	18.8	24.8	19.3	22.5	19.8	22.8	15.8	18.7	22.3				
e4	1	27.1^{c}	**80.9^{cb}**	30.0^{c}	57.4^{bc}	41.4^{bc}	33.4^{c}	11.5^{c}	15.5^{c}	90.4^{bc}	< 0.0001	< 0.0001	0.009	0.020
	5	18.5^{c}	**240.8^{a}**	34.0^{c}	92.8^{bc}	38.1^{bc}	43.5^{bc}	10.9^{c}	22.6^{c}	159.6^{ab}				
e5	1	3.3	4.4	23.7	35.5	17.3	153.3	25.1	220.1	70.0	NS	0.022	NS	NS
	5	2.3	18.0	23.1	10.2	4.0	10.6	28.3	199.9	25.9				
e6	1	1481.5	306.0	2191.3	847.7	5039.8	3013.8	2723.9	3579.3	259.9	0.008	0.0002	NS	NS
	5	1960.8	440.2	3281.7	236.8	4121.7	2986.0	3100.2	3520.3	210.1				
e7	1	2.2^{b}	5.0^{b}	11.0^{b}	8.5^{b}	43.7^{ab}	18.6^{b}	44.3^{ab}	80.0^{ab}	16.2^{b}	0.013	0.001	NS	NS
	5	1.6^{b}	18.7^{b}	7.2^{b}	6.3^{b}	4.8^{b}	3.2^{b}	6.0^{b}	162.0^{a}	13.5^{b}				
e8	1	9.0^{b}	29.3^{ab}	57.9^{a}	53.0^{ab}	11.9^{ab}	17.4^{ab}	24.9^{ab}	38.1^{ab}	31.7^{ab}	0.003	0.001	NS	NS
	5	12.8^{ab}	47.7^{ab}	21.0^{ab}	42.8^{ab}	15.5^{ab}	11.3^{ab}	16.6^{ab}	36.5^{ab}	43.1^{ab}				
e9	1	237.9^{ab}	135.0^{ab}	114.8^{ab}	62.3^{b}	159.0^{ab}	186.2^{ab}	306.4^{ab}	512.0^{a}	29.5^{b}	0.008	0.001	NS	NS

Code	Day	Approximate quantity[a]									P[p]	P[c]		
		RG	Hapil	RG010	RG086	RG098	RG100	RG126	RG164	RG169		Genotype	Day	Interaction
	5	244.7^{ab}	303.3^{ab}	125.7^{ab}	44.3^{b}	103.5^{ab}	61.6^{b}	282.1^{ab}	303.9^{ab}	19.6^{b}				
e10	1	1694.7^{ab}	178.2^{b}	1905.3^{ab}	3119.1^{ab}	4077.9^{ab}	5404.8^{ab}	9538.4^{a}	6240.9^{ab}	1642.5^{ab}	0.019	0.005	0.041	NS
	5	1107.7^{ab}	570.1^{b}	3729.8^{ab}	277.7^{b}	1108.9^{ab}	2259.3^{ab}	4494.4^{ab}	4721.9^{ab}	723.7^{b}				
e11	1	91.7	53.4	189.0	261.4	316.1	613.2	228.0	308.5	219.7	NS	NS	NS	NS
	5	140.2	106.6	532.4	71.2	145.9	227.5	193.4	362.1	131.9				
e12	1	10.4	0.8	9.4	7.0	36.4	15.5	15.2	24.7	3.5	0.032	0.002	NS	NS
	5	19.5	2.1	13.5	1.2	28.5	33.4	18.4	21.7	1.9				
e13	1	261.0	81.2	150.8	306.5	694.4	836.4	438.2	231.9	60.2	NS	NS	NS	NS
	5	259.7	161.4	320.6	57.9	1014.5	295.6	391.6	304.8	134.9				
e14	1	11.6^{b}	33.1^{b}	159.8^{b}	137.1^{b}	114.7^{b}	338.6^{ab}	125.1^{b}	492.6^{ab}	272.0^{ab}	0.037	0.012	NS	NS
	5	3.7^{b}	149.5^{b}	118.2^{b}	33.1^{b}	37.3^{b}	30.0^{b}	144.2^{b}	1730.8^{a}	82.1^{b}				
e15	1	39.3^{c}	75.9^{abc}	171.1^{ab}	171.7^{ab}	112.2^{abc}	88.4^{abc}	104.4^{abc}	117.1^{abc}	188.5^{a}	0.001	0.002	0.001	NS
	5	36.3^{c}	94.2^{abc}	74.2^{abc}	111.7^{abc}	60.4^{b}c	41.9^{c}	84.1^{abc}	95.9^{abc}	81.5^{abc}				
e16	1	22.2^{b}	46.0^{ab}	151.1^{a}	41.4^{ab}	44.0^{ab}	35.9^{ab}	74.0^{ab}	72.2^{ab}	78.5^{ab}	0.027	0.003	NS	NS
	5	27.0^{b}	80.8^{ab}	85.6^{ab}	40.5^{ab}	34.0^{ab}	13.7^{b}	64.2^{ab}	104.0^{ab}	58.8^{ab}				
e17	1	42.0^{e}	66.8^{de}	117.5^{cde}	233.8^{a}	93.3^{cde}	139.2^{bcd}	110.8^{cde}	126.7^{cd}	**210.9^{ab}**	< 0.0001	< 0.0001	< 0.0001	0.002
	5	62.6^{de}	86.3^{cde}	88.1^{cde}	154.1^{abc}	82.0^{cde}	73.8^{cde}	89.5^{cde}	64.7^{de}	**108.8^{cde}**				
e18	1	3655.2^{ab}	185.9^{b}	1175.1^{ab}	1151.0^{ab}	3674.6^{ab}	2978.6^{ab}	3899.1^{ab}	1732.3^{ab}	2059.6^{ab}	0.002	0.0001	NS	NS
	5	4293.5^{a}	302.6^{b}	1768.7^{ab}	302.4^{b}	2525.4^{ab}	2420.4^{ab}	2128.8^{ab}	1100.9^{ab}	907.4^{ab}				
e19	1	33.1	29.8	46.8	37.7	46.9	54.7	40.4	75.6	58.6	0.022	0.002	NS	NS

Code	Day	Approximate quantity[a]									P^p	P^c		
		RG	Hapil	RG010	RG086	RG098	RG100	RG126	RG164	RG169		Genotype	Day	Interaction
	5	47.2	29.0	26.9	32.4	36.9	38.1	20.5	77.9	65.6				
e20	1	45.1	11.9	72.8	30.6	102.1	196.1	261.9	208.7	11.7	0.013	0.001	NS	NS
	5	120.0	16.9	107.5	9.1	72.0	94.6	139.8	164.1	16.8				
e21	1	1982.4	132.8	2982.2	3828.3	3885.3	11178.4	15645.7	8392.9	8697.5	NS	0.051	0.012	NS
	5	2272.2	349.1	3554.3	362.3	1410.3	2976.3	5971.0	2454.2	2670.1				
e22	1	1515.3^{c}	2096.2^{bc}	4794.1^{a}	2848.9^{abc}	4079.9^{ab}	3554.9^{abc}	2845.0^{abc}	2700.1^{abc}	4934.4^{a}	0.0001	< 0.0001	0.040	NS
	5	1849.2^{bc}	2017.4^{bc}	3679.7^{abc}	2860.7^{abc}	3582.9^{abc}	2700.7^{abc}	3314.3^{abc}	2026.4^{bc}	3151.6^{abc}				
e23	1	5.7^{abc}	7.9^{abc}	18.1^{a}	14.1^{abc}	8.9^{abc}	9.1^{abc}	15.4^{ab}	8.7^{abc}	16.9^{a}	0.001	0.013	< 0.0001	NS
	5	1.3^{bc}	5.2^{abc}	8.9^{abc}	7.9^{abc}	8.0^{abc}	0.6^{c}	5.8^{abc}	4.1^{abc}	4.1^{abc}				
e24	1	48.1	7.4	28.4	43.0	43.9	67.4	87.2	91.6	22.2	NS	0.025	NS	NS
	5	79.4	0.0	35.9	0.0	35.0	32.8	50.2	65.4	50.4				
e25	1	$67.6b^{c}$	$65.2b^{c}$	107.7^{abc}	75.3^{abc}	54.4^{bc}	**214.0^{a}**	191.9^{ab}	214.5^{a}	160.0^{abc}	< 0.0001	0.0002	< 0.0001	NS
	5	43.3^{c}	44.7^{c}	49.2^{bc}	57.6^{bc}	30.1^{c}	**36.8^{c}**	104.9^{abc}	116.2^{abc}	80.5^{abc}				
e26	1	917.8^{ab}	140.8^{b}	325.2^{b}	353.7^{b}	1083.7^{ab}	1443.2^{ab}	3709.8^{a}	655.4^{ab}	97.1^{b}	0.012	0.001	NS	NS
	5	698.2^{ab}	113.2^{b}	1181.6^{ab}	76.0^{b}	862.8^{ab}	1051.7^{ab}	2122.3^{ab}	755.5^{ab}	77.4^{b}				
e27	1	1183.4	29.4	73.3	36.2	50.6	671.7	939.6	392.9	196.5	NS	0.521	0.039	NS
	5	35.0	8.1	59.0	5.3	12.8	41.5	186.5	46.3	23.8				
e28	1	74.4	23.4	165.4	87.6	95.0	489.8	105.5	145.2	80.9	NS	NS	NS	NS
	5	79.8	22.1	193.6	32.9	67.1	52.9	88.3	49.1	33.9				
e29	1	34.7^{ab}	15.9^{b}	33.6^{ab}	67.5^{ab}	78.5^{ab}	64.2^{ab}	120.2^{a}	41.5^{ab}	32.2^{ab}	0.005	0.022	0.0003	NS

Code	Day	Approximate quantity[a]									P[p]	P[c]		
		RG	Hapil	RG010	RG086	RG098	RG100	RG126	RG164	RG169		Genotype	Day	Interaction
	5	22.1^{b}	16.4^{b}	30.2^{b}	23.7^{b}	18.9^{b}	15.2^{b}	43.2^{ab}	16.5^{b}	19.0^{b}				
e30	1	83.8^{c}	144.1^{abc}	200.5^{abc}	251.3^{ab}	185.1^{abc}	134.1a^{bc}	148.2^{abc}	133.5^{abc}	274.0^{a}	0.0001	< 0.0001	NS	NS
	5	97.1^{bc}	200.7^{abc}	95.6^{bc}	261.3^{a}	186.4^{abc}	98.3b^{c}	177.4^{abc}	81.7^{c}	181.2^{abc}				
e31	1	2894.3^{b}	4498.7^{ab}	4867.5^{ab}	5020.1^{ab}	5926.6^{ab}	5110.8^{ab}	4067.8^{ab}	4932.2^{ab}	7617.8^{a}	0.036	0.003	NS	NS
	5	2927.9^{b}	5376.9^{ab}	4318.6^{ab}	6376.3^{ab}	5689.8^{ab}	4919.8^{ab}	5015.4^{ab}	4090.9^{ab}	6296.0^{ab}				
Terpenes														
t1	1	32.0^{c}	37.7^{c}	127.3^{abc}	87.2^{bc}	154.4^{ab}	35.7^{c}	44.4^{c}	34.1^{c}	39.5^{c}	< 0.0001	< 0.0001	NS	NS
	5	21.7^{c}	34.8^{c}	109.3^{abc}	44.9^{c}	207.9^{a}	56.6^{bc}	40.0^{c}	30.7^{c}	29.9^{c}				
t2	1	51.9^{b}	52.0^{b}	124.3^{ab}	100.6^{ab}	182.7^{ab}	72.5^{b}	60.8^{b}	49.8^{b}	64.8^{b}	0.002	< 0.0001	NS	NS
	5	41.1^{b}	104.6^{ab}	140.6^{ab}	52.8^{b}	221.2^{a}	81.1^{ab}	61.8^{b}	80.3^{ab}	89.7^{ab}				
t3	1	**11.1^{bcd}**	**3.4^{d}**	8.8^{cd}	9.4^{cd}	11.1^{bcd}	10.4^{cd}	12.0^{bcd}	13.9^{bcd}	10.2^{cd}	< 0.0001	NS	< 0.0001	NS
	5	**47.6^{a}**	**31.1^{abc}**	33.4^{abc}	23.1^{abcd}	25.4^{abcd}	32.1^{abc}	36.4^{ab}	33.0^{abc}	25.8^{abcd}				
t4	1	19.2^{c}	21.6^{c}	73.7^{abc}	49.6^{bc}	95.7^{ab}	19.1^{c}	24.4^{c}	17.1^{c}	19.5^{c}	< 0.0001	< 0.0001	NS	NS
	5	10.7^{c}	17.6^{c}	62.5^{abc}	24.7^{c}	128.7^{a}	29.1^{bc}	21.0^{c}	14.3^{c}	14.3^{c}				
t5	1	32.4^{bc}	28.4^{bc}	76.8^{abc}	67.0^{abc}	148.6^{a}	26.4^{bc}	38.7^{bc}	20.4^{bc}	19.6^{c}	0.0002	< 0.0001	NS	NS
	5	15.8^{c}	28.1^{bc}	68.9^{abc}	20.4^{bc}	119.2^{ab}	26.7^{bc}	22.7^{bc}	15.9^{c}	13.4^{c}				
t6	1	461.6^{c}	597.8^{c}	2191.4^{abc}	1616.9^{abc}	2801.3^{ab}	662.4^{c}	951.8^{bc}	627.5^{c}	759.2^{c}	< 0.0001	< 0.0001	NS	NS
	5	390.5^{c}	490.3^{c}	1769.7^{abc}	605.1^{c}	3019.0^{b}	689.0^{c}	639.0^{c}	504.6^{c}	497.0^{c}				
t7	1	53.9^{b}	54.0^{b}	143.2^{ab}	154.6^{ab}	265.6^{a}	67.3^{ab}	69.8^{ab}	48.2^{b}	54.6^{b}	0.002	< 0.0001	NS	NS
	5	40.1^{b}	43.4^{b}	145.9^{ab}	48.5^{b}	211.4^{ab}	73.2^{ab}	45.8^{b}	43.7^{b}	38.5^{b}				

Code	Day	Approximate quantity[a]									P[p]	P[c]		
		RG	Hapil	RG010	RG086	RG098	RG100	RG126	RG164	RG169		Genotype	Day	Interaction
t8	1	3.8[b]	3.7[b]	22.0[ab]	21.4[ab]	37.6[a]	5.9[b]	2.9[b]	3.1[b]	5.4[b]	0.0015	0.0004	0.003	NS
	5	0.0[b]	0.0[b]	8.0[ab]	0.0[b]	19.5[ab]	0.0[b]	0.0[b]	0.0[b]	0.0[b]				
Alcohol														
a1	1	75.0[c]	107.9[bc]	297.4[ab]	118.1[bc]	104.5[bc]	94.6[c]	118.9[bc]	91.6[c]	139.7[bc]	0.0002	< 0.0001	0.001	NS
	5	135.4[bc]	113.9[bc]	354.7[a]	165.0[abc]	194.9[abc]	182.7[abc]	205.6[abc]	169.5[abc]	198.7[abc]				
a2	1	50.5	30.4	48.8	64.6	65.6	133.2	140.0	99.1	58.0	NS	NS	0.004	NS
	5	40.0	29.8	42.3	21.5	35.1	36.5	50.7	59.7	21.0				
a3	1	31.3	24.5	16.7	22.7	8.6	41.4	23.7	20.2	13.7	NS	NS	0.001	NS
	5	16.7	7.4	11.4	0.0	0.0	0.0	4.6	11.1	0.0				
a4	1	107.2	228.2	226.0	189.0	337.4	126.7	132.3	103.8	196.2	0.027	0.010	0.029	NS
	5	160.1	187.7	335.6	282.5	269.9	233.6	229.7	140.5	296.3				
aldehydes														
ald1	1	1.4	4.9	1.5	2.4	1.9	0.8	2.3	3.0	1.8	NS	0.047	NS	NS
	5	2.5	2.8	2.7	4.0	1.6	2.0	5.1	4.0	1.6				
ald2	1	25.7[ab]	52.0[a]	29.4[ab]	35.6[ab]	22.5[b]	40.2[ab]	29.8[ab]	31.8[ab]	36.5[ab]	0.001	< 0.0001	NS	NS
	5	31.6[ab]	52.0[a]	24.4[b]	46.5[ab]	23.1[b]	27.6[ab]	31.4[ab]	20.9[b]	31.3[ab]				
ald3	1	189.9[b]	279.7[ab]	629.6[ab]	319.7[ab]	374.6[ab]	458.0[ab]	504.7[ab]	323.2[ab]	479.0[ab]	0.006	0.001	NS	NS
	5	241.0[ab]	296.8[ab]	335.8[ab]	284.3[ab]	361.1[ab]	561.5[ab]	412.4[ab]	188.0[b]	635.8[a]				
ald4	1	54.0[a]	41.7[ab]	34.5[ab]	27.6[ab]	5.3[ab]	43.4[ab]	50.4[ab]	23.3[ab]	36.1[ab]	0.012	0.008	0.002	NS
	5	28.2[ab]	35.4[ab]	0.0[b]	19.8[ab]	8.6[ab]	23.5[ab]	35.9[ab]	15.3[ab]	14.6[ab]				

Code	Day	Approximate quantity[a]									P[p]	P[c]		
		RG	Hapil	RG010	RG086	RG098	RG100	RG126	RG164	RG169		Genotype	Day	Interaction
ald5	1	177.9	118.8	101.2	85.0	91.0	122.7	258.5	230.0	81.7	NS	0.021	NS	NS
	5	106.1	94.8	89.6	66.8	58.7	86.6	202.9	145.3	73.4				
ald6	1	2440.3^{bc}	3856.0^{abc}	4970.9^{abc}	4683.8^{abc}	4313.5^{abc}	3678.5^{abc}	3471.9^{abc}	2793.7^{bc}	4389.9^{abc}	0.001	0.0003	NS	NS
	5	2427.9^{bc}	3215.9^{abc}	3376.2^{abc}	3684.1^{abc}	4461.5^{abc}	5553.7^{ab}	4226.5^{abc}	2236.2^{c}	6312.1^{a}				
ald7	1	41.9^{b}	76.8^{ab}	99.5^{ab}	180.6^{a}	61.4^{b}	57.3^{b}	69.9^{b}	75.7^{ab}	110.6^{ab}	0.004	0.0005	NS	NS
	5	47.4^{b}	71.9^{b}	53.9^{b}	121.8^{ab}	68.8^{b}	98.3^{ab}	69.7^{b}	54.9^{b}	106.6^{ab}				
ald8	1	61.3^{ab}	67.2^{ab}	81.2^{ab}	68.0^{ab}	87.0^{ab}	94.4^{ab}	66.7^{ab}	49.9^{ab}	90.0^{ab}	0.035	0.004	NS	NS
	5	85.4^{ab}	72.6^{ab}	65.2^{ab}	59.4^{ab}	96.7^{ab}	118.6^{ab}	77.7^{ab}	39.6^{b}	123.2^{a}				
ald9	1	29.1^{a}	15.1^{ab}	7.2^{b}	6.9^{b}	4.2^{b}	15.6^{ab}	12.4^{ab}	10.2^{ab}	13.5^{ab}	0.001	0.001	0.0004	NS
	5	14.5^{ab}	6.1^{b}	0.0^{b}	5.4^{b}	0.0^{b}	0.0^{b}	9.0^{ab}	2.8^{b}	9.7^{ab}				
Furanones														
f1	1	95.5	12.3	33.0	61.8	183.9	144.3	35.8	103.8	69.7	NS	0.510	NS	NS
	5	25.2	14.3	68.0	35.8	10.7	68.1	44.3	48.8	88.5				
f2	1	780.1^{ab}	572.9^{ab}	480.9^{b}	968.3^{ab}	567.7^{ab}	3305.2^{a}	725.1^{ab}	926.8^{ab}	2717.5^{ab}	0.011	0.001	NS	NS
	5	669.7^{ab}	1087.9^{ab}	857.2^{ab}	1190.6^{ab}	720.5^{ab}	1223.5^{ab}	618.6^{ab}	1265.8^{ab}	2614.4^{ab}				
Carboxylic acids														
c1	1	81.2^{ab}	119.2^{ab}	51.1^{b}	94.7^{ab}	53.5^{b}	164.9^{ab}	47.7^{b}	135.8^{ab}	284.5^{ab}	0.006	0.0003	NS	NS
	5	61.2^{b}	249.8^{ab}	65.0^{b}	140.7^{ab}	37.8^{b}	153.5^{ab}	34.0^{b}	209.2^{ab}	385.0^{b}				
c2	1	110.9^{bc}	74.2^{bc}	40.3^{c}	93.1^{bc}	142.2^{abc}	156.7^{abc}	153.4^{abc}	232.0^{ab}	162.3^{abc}	0.0003	< 0.0001	NS	NS
	5	105.9^{bc}	135.2^{bc}	71.2^{bc}	85.8^{bc}	82.1^{bc}	93.2^{bc}	126.4^{bc}	305.5^{a}	176.6^{abc}				

Code	Day	Approximate quantity[a]									P[p]	P[c]		
		RG	Hapil	RG010	RG086	RG098	RG100	RG126	RG164	RG169		Genotype	Day	Interaction
c3	1	523.1^{ab}	205.1^{ab}	80.4^{b}	307.1^{ab}	117.5^{b}	368.4^{ab}	194.9^{ab}	76.5^{b}	536.3^{ab}	0.001	< 0.0001	NS	NS
	5	747.7^{a}	354.5^{ab}	132.6^{ab}	332.6^{ab}	94.5^{b}	591.1^{ab}	101.3^{b}	96.7^{b}	675.4^{ab}				
c4	1	1357.4^{ab}	119.1^{b}	223.2^{b}	590.9^{ab}	906.8^{ab}	868.4^{ab}	1003.8^{ab}	497.1^{ab}	703.4^{ab}	0.002	0.0003	NS	NS
	5	1901.5^{a}	155.9^{b}	744.3^{ab}	381.7^{ab}	596.5^{ab}	1876.1^{a}	599.0^{ab}	546.0^{ab}	640.3^{ab}				
c5	1	21.9	42.4	7.8	7.1	8.4	11.7	9.7	7.0	10.5	NS	NS	NS	NS
	5	13.8	5.4	8.6	9.3	10.2	20.3	8.4	11.4	15.4				
Ketones														
k2	1	12.5	13.7	26.6	22.5	27.2	120.2	45.3	119.6	31.0	NS	NS	NS	NS
	5	7.3	11.2	25.6	16.3	16.1	8.7	60.4	48.4	19.1				
k3	1	11.6^{ab}	8.4^{ab}	11.4^{ab}	12.7^{ab}	5.4^{b}	11.3^{ab}	24.5^{ab}	13.4^{ab}	19.3^{ab}	0.024	0.048	0.006	NS
	5	26.7^{a}	14.3^{ab}	21.1^{ab}	15.4^{ab}	12.9^{ab}	20.3^{ab}	20.6^{ab}	17.5^{ab}	15.7^{ab}				

[a] Estimated quantities (ng) collected from the headspace of 5 g of strawberry pulp, calculated by comparison with 25 µl of 3-heptanol (50 ppm) used as internal standard; means not labelled with the same letters are significantly different (p<0.05). Those compounds significantly different between the two shelf life days are marked in bold. **[b]** Probability, as obtained from one-way ANOVA, that there is a difference between means; NS: no significant difference between means (P<0.05). **[c]** Probability, as obtained from two-way ANOVA, that there is a difference between means; NS: no significant difference between means (P<0.05). Superscript letters for each compound indicate differing levels of significance for each respective genotype (ANOVA Tukey's HSD test; (p≤0.05)). Codes on table refer to compound codes in Table 5.3.

5.3.1.6.1 *Esters*

Esters (acetates and non-acetate esters) were the most abundant group, in terms of the number of detected compounds and quantities, of volatiles among the nine genotypes. It comprised 74.4 % and 68.2 %, for day 1 and day 5, respectively, of the total volatiles collected from the headspace of the nine strawberry genotypes. This further confirms that the ester group is the most abundant class of compounds in strawberry aroma. Acetate and butanoate esters were the two main groups of compound identified in RGxH progeny lines, which is consistent with previous studies in raspberry fruits (Giuggioli et al., 2015). The most abundant esters identified were ethyl propanoate (both days), methyl 2-methylbutanoate (day 1), pentyl acetate (day 1), 2-methylpropyl butanoate (both days), ethyl hexanoate (both days), and (E)-2-hexen-1-ol acetate (both days). However, the distribution of methyl and ethyl esters, two of major esters in strawberry, was variable and appears to be genotype-dependant (Table 5.4).

Even though esters were the most numerous and as a group provided the predominant aroma character to strawberry, the major esters were found to be ethyl acetate, methyl butanoate, ethyl butanoate, methyl 2-methylbutanoate, butyl acetate, methyl hexanoate, ethyl hexanoate, ethyl isovalerate (Azodanlou et al., 2003; Bood and Zabetakis, 2002; Du et al., 2011a; El Hadi et al., 2013; Hakala et al., 2002; Jetti et al., 2007; Miszczak et al., 1995; Schwieterman et al., 2014; Song and Forney, 2008; Zabetakis and Holden, 1997). These esters contribute to the

fruity and floral notes of the strawberry aroma (Forney et al., 2000; Jetti et al., 2007; Pelayo et al., 2003; Song and Forney, 2008). However, their concentration is known to be cultivar specific (El Hadi et al., 2013; Jetti et al., 2007).

The highest levels of esters recorded were ethyl hexanoate (15645.7 and 11178.4 ng (collected from the headspace of 5 g of strawberry pulp) for RG126-day 1 and RG100-day 1, respectively), methyl 2-methylbutanoate (9538.4 ng for RG126-day 1), and (E)-2-hexen-1-ol acetate (7617.8 ng for RG169-day 1). Most of these highest values were recorded for samples of day 1. Oz et al. (2016) had the ethyl hexanoate as the main ester in six out of eight different varieties. Masses related to esters were varied based on genotype and day/storage which ranged from 0 to 15645 ng. 2-methylpropyl butanoate, one of the major volatile compounds in strawberry, reached a concentration of 15645 ng (33 % of esters content for RG126-day 1).

Esters are known for their fruity odour note, therefore the increase of these compounds during storage may indicate that this fruity note was maintained during storage. Across the parental lines, Hapil showed an increase during storage for 20 out of 31 esters, while RG showed divergent trends between volatiles (Table 5.4). Hapil showed a significant increase during shelf life storage for isopropyl acetate. However, the genotype effect was abundant over the days/storage effect as most of the ester compounds were significantly different between genotypes for all esters ($p \leq 0.05$), except for methyl propanoate, butyl

acetate, isopropyl butanoate and octyl acetate. However, 10 out of 31 esters were significantly different between days/storage. Pentyl acetate was statistically significant across storage for RG169, while benzyl acetate was statistically significant across storage for RG100. For isopropyl acetate, five F1 offspring lines (RG010, RG086, RG100, RG164, and RG169) had higher amount at day 5, whereas for ethyl butanoate, 3-methylbutyl acetate, pentyl acetate, ethyl hexanoate, hexyl acetate, heptyl acetate, benzyl acetate, ethyl octanoate, and methyl salicylate the ester amount per each compound separately was remarkably lower at day 5 (Table 5.4).

In general, esters content of 28 compounds at day 1 were higher comparing to day 5. Forney et al. (2000) and Miszczak et al. (1995) reported that volatile compounds (mainly esters) increased during post-harvest storage at 15 °C after 4 days. Similar increases in volatile content were reported by Forney and Jordan (1995). During 5 days at 1 °C and 2 days at 15 °C, volatile content of 'Kent', 'Annapolis', 'Micmac', 'Cavendish', and 'Honeoye' fruit were 5.7, 1.9, 1.7, 1.4, and 1.3 times as high, respectively. Such increase could be explained by the result of the increased synthesis and accumulation of ester compounds in the fruit tissues during the first days of storage (Miszczak et al., 1995). In addition, an increase in some ester compounds during postharvest storage may be partially attributed to the water loss of the fruit during storage (Miszczak et al., 1995). Storage temperature influences strawberry volatile production. This indicates that the

influence of post-harvest storage on volatile compounds (mainly esters) is temperature-dependant (Forney et al., 2000). In this experiment, ethyl hexanoate decreased during storage to a greater extent comparing to other esters. After 4 days at 4 °C, ethyl hexanoate content of nine strawberry genotypes fruit decreased from 9.6 % to 3.7 % of total volatiles. After 5 days of postharvest storage, both (E)-2-hexen-1-ol acetate and ethyl butanoate were the highest concentration. Miszczak et al. (1995) found that during 10 days of postharvest storage at 15 °C, the major esters produced pink and red berries were methyl and ethyl butanoate.

5.3.1.6.2 *Terpenes*

Detectable levels of terpenes were presented among the nine genotypes. They account for 4.2 % and 4.5 %, for day 1 and day 5, respectively, of the total volatiles collected from the headspace of the nine strawberry genotypes. Eight terpenes were identified including beta-pinene, d-limonene, eucalyptol, beta-ocimene alpha-terpinolene, linalool, alpha-terpineol, and cis-geraniol (Table 5.4). Among them, linalool, common fruit volatile with a floral/rose odour (Rowan, 2011; Zhang et al., 2009), was the most abundant terpene for both post-harvest days. Previously, linalool was reported in many fruits including grapes (Mateo and Jiménez, 2000) and strawberry (Azodanlou et al., 2003; Mishra and Kar, 2014; Zabetakis and Holden, 1997; Zhang et al., 2009; Zorrilla-Fontanesi et al., 2012). In strawberry, Mishra and Kar (2014) reported a significant decrease of terpenes with the increase in the storage period at 5 ° C for 9 days. Also in grapes, it was

present at a low levels in early stages, reaching its highest amounts for red 3/4 and red 4/4 stages, and then slightly decreased (Mateo and Jiménez, 2000). In strawberry, linalool was reported to be one of the major volatile compounds linked to strawberry flavour (Azodanlou et al., 2003; Ménager et al., 2004; Miszczak et al., 1995; Schwieterman et al., 2014)

5.3.1.6.3 *Alcohols*

Alcohols, known as a green component as they are the main group identified in immature strawberry fruits by Ménager et al. (2004), contribute to flavour and aroma of the fruits. They are known as precursors for ester synthesis (Song and Forney, 2008). They accounted for 1.6 % and 2.1 %, for day 1 and day 5, respectively, of the total volatiles collected from the headspace of the nine strawberry genotypes. Four alcohols were identified including 1-hexanol, benzaldehyde, 1-octanol, and (E)-2-hexen-1-ol (Table 5.4). Among them, 1-hexanol and (E)-2-hexen-1-ol were the most abundant compounds. These two abundant compounds increased during the post-harvest storage/day. Within strawberries, these two abundant compounds were reported previously in some studies (Forney et al., 2000; Hakala et al., 2002; Schwieterman et al., 2014; Zorrilla-Fontanesi et al., 2012).

5.3.1.6.4 *Aldehydes*

Besides esters, terpenes, and alcohols, nine aldehydes were identified in the samples (Table 5.4). They are known as a green component as they are the main

group identified in immature strawberry fruits by Ménager et al. (2004). They were comprised 12.6 % and 16 %, for day 1 and day 5, respectively, of the total volatiles collected from the headspace of the nine strawberry genotypes. 3-Methylbutanal, pentanal, hexanal, heptanal, nonanal, (E)-2-hexenal, (E,E)-2,4-hexadienal, (E)-2-heptenal, and (Z)-2-decenal were the major aldehydes identified in this study. The highest levels of aldehydes recorded was (E)-2-hexenal, which known to play a role in plant defence (Ceuppens et al., 2015). It is also known to have antimicrobial and antifungal characteristics (Kishimoto et al., 2008). Hexanal and (E)-2-hexenal known for their green/grass and unripe notes in strawberry aroma (El Hadi et al., 2013; Lambert et al., 1999). The level of (E)-2-hexenal varied based on the availability of (E)-3-hexenal, which is known as the precursor of the intense green odour compound (E)-2-hexenal (Larsen and Poll, 1992; A.G. Pérez et al., 1997; Ulrich et al., 1997). This means that any increase or decrease for the level of (E)-2-hexenal during storage depends on the quick/slow conversion of (Z)-3-hexenal to (E)-2-hexenal. Previously, hexanal and (E)-2-hexenal were reported as the major aldehyde compounds in raspberries at harvest (Giuggioli et al., 2015).

5.3.1.6.5 *Furanones*

Although little is known about their biosynthesis and metabolism (Giuggioli et al., 2015), detectable levels of furanones were also present. They account for 3.6 % and 4.1 %, for day 1 and day 5, respectively, of the total volatiles collected

from the headspace of the nine strawberry genotypes. Two furanones were identified including 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) and 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifurane). They are considered to be the two most important furanones in strawberry aroma (Jetti et al., 2007; Ménager et al., 2004; Mishra and Kar, 2014; Song and Forney, 2008; Zorrilla-Fontanesi et al., 2012). Special attention was paid to furanones during ripening in four varieties of strawberry by Perez et al. (1996) and Ménager et al. (2004). Furaneol is not stable and its degradation depends on pH and temperature. Mesifurane is more stable than furaneol. Mesifurane and furaneol content increase with the ripening (Jetti et al., 2007). At high concentrations, furaneol imparts caramel and sweet notes and fruity notes at lower concentrations, while mesifurane has been reported as having a more burnt, sherry-like, or fusty aroma (Jouquand et al., 2008; Larsen and Poll, 1992; Perez et al., 1996; Zorrilla-Fontanesi et al., 2012).

Both compounds (mesifurane and furaneol) were found to be statistically non-significant with storage time (Table 5.4). However, some genotypes showed an increase during storage, while others showed a decrease which may indicate the genotype-dependant, as previously reported by Perez et al. (1996) who found that the level of furanones was different between four strawberry cultivars. Previously, the level of furaneol was higher in overripe fruits comparing to ripe fruit (Pelayo-Zaldivar et al., 2007), which could suggest that the furaneol synthesis (and other

furanones) is taken place even after the fruit attained the full ripe level (Perez et al., 1996).

5.3.1.6.6 *Carboxylic acids*

Five carboxylic acids were identified in the samples (Table 5.4). They comprised more than 3.3 % and 5 %, for day 1 and day 5, respectively, of the total volatiles collected from the headspace of the nine strawberry genotypes. These compounds include acetic acid, 2-methylpropanoic acid, butanoic acid, hexanoic acid, and octanoic acid. Among them, hexanoic acid was the highest level recorded. All five acid compounds were previously identified in cultivated (*Fragaria x ananassa*) strawberry (Ménager et al., 2004; Zabetakis and Holden, 1997). Compounds formed from organic acids such as acetic acid, butanoic acid and hexanoic acid were the most important ones in cultivated strawberries (Modise, 2008). Octenoic acid, hexanoic acid, and octanoic acid were the most important ones in pineapple fruits (El Hadi et al., 2013). However, all acid compounds were found to be statistically non-significant with storage time (Table 5.4). Previously, acid compounds were found in white strawberry fruits and their concentrations increased significantly during maturation, then slightly decreased (Ménager et al., 2004).

5.3.1.6.7 *Ketones*

Two ketones (3-octanone and acetophenone) were identified in the samples (Table 5.4). They comprised 0.2 % and 0.1 %, for day 1 and day 5, respectively,

of the total volatiles collected from the headspace of the nine strawberry genotypes for both days. 3-Octanone and acetophenone were the major ketones identified in this study. Acetophenone, which was previously reported in strawberry studies (Zabetakis and Holden, 1997; Zorrilla-Fontanesi et al., 2012), functions as a repellent to herbivores (Ceuppens et al., 2015; Suchet et al., 2011).

5.3.1.6.8 *Principal Component Analysis*

Principal component analysis was used to visualise graphically the differences in volatiles in the nine genotypes at two different post-harvest days (day 1 and day 5; Figure 5.2). The first two principal components explained 41.82 % of the variation in the data (Figure 5.2). Across the variables (volatiles) shown in Figure 5.2.a, the majority of esters (14 ester compounds; above right) and terpenes (7 terpene compounds; above left) were clustered separately to the other volatile groups (Figure 5.2.a). Whereas, other groups (alcohols, ketones, aldehydes, furanones, and carboxylic acids) were mostly distributed over the PCA, therefore they might not have a large variance. The first axis discriminates the majority of esters (above right) and 2 alcohols (1-hexanol and (E)-2-hexen-1-ol; above left). Such negative correlation between esters and alcohols is expected, as alcohol serving as precursors for ester synthesis (Song and Forney, 2008). Previously, Zorrilla-Fontanesi et al. (2012) reported a negative correlation between esters and alcohols, particularly between 1-hexanol (18) and butyl hexanoate (r=20.30) or octyl hexanoate (r=20.29).

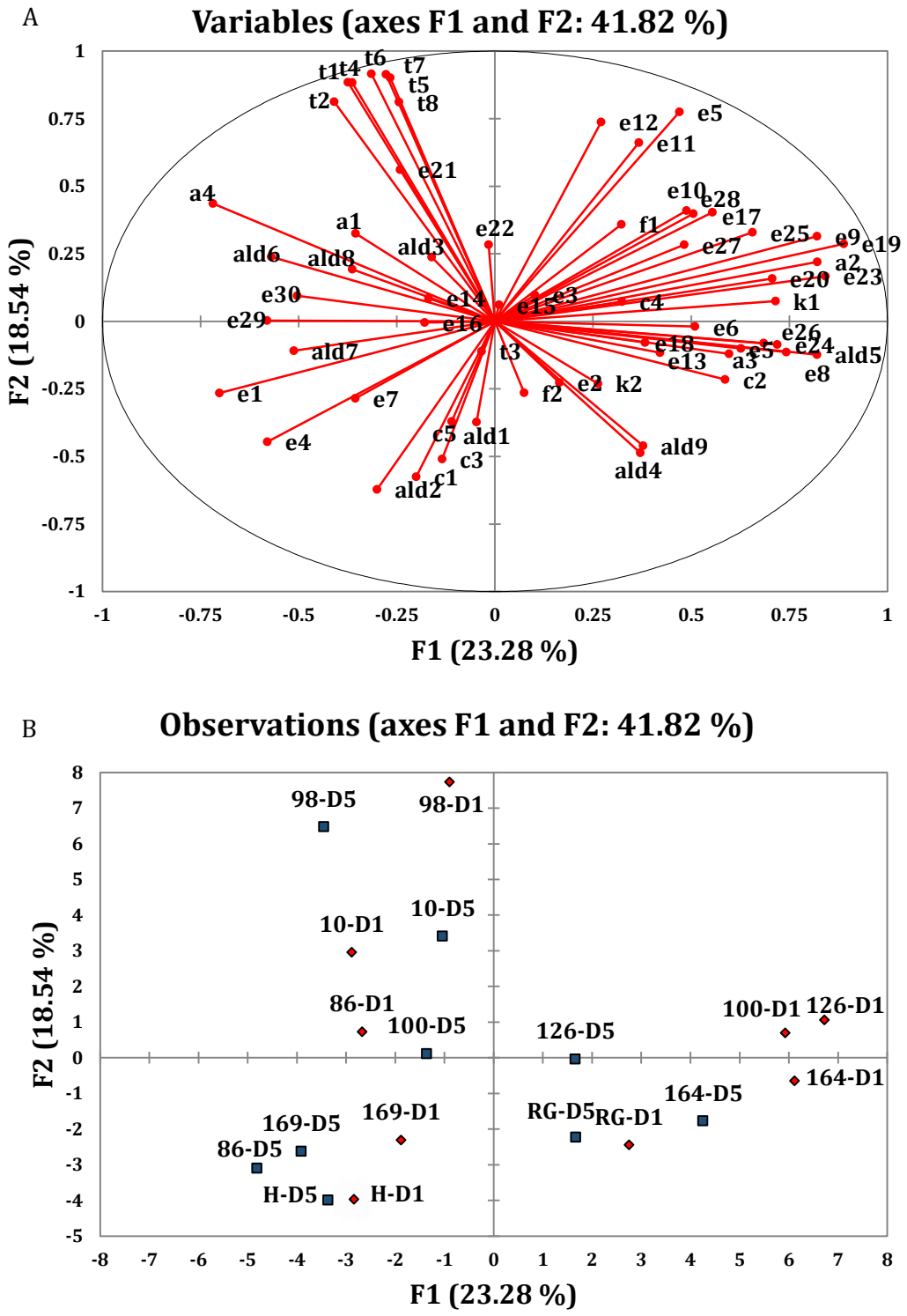


Figure 5.2. Principal component analysis of the nine genotypes of RGxH progeny measured at two different shelf life days showing correlation with volatile compounds. Data plotted are the differences between day 1 and day 5. (A) Distribution of variables (codes on plot refer to compound codes in Table 5.3). (B) Projection of the samples; day 1 samples are shown in red dots, and day 5 samples are shown in blue dots.

Positive and negative correlations were noted between the volatile compounds of each single group (Figure 5.3; Pearson correlation coefficients (n-1); $P<0.05$). Of these, the highest positive correlations were found between methyl butanoate (e6) and isopropyl butanoate (e13; $r=0.934$) as well as between terpenes, whose correlation coefficients ranged between 0.933 and 0.999 (t1, t4, t5, t6, t7 and t8; Figure 5.2). As previously reported in strawberry (Zorrilla-Fontanesi et al., 2012), negative correlations were found between alcohols and esters, as for instance between (E)-2-hexen-1-ol (a4) and five esters include isopropyl acetate (e4), 2-methylpropyl acetate (e8; $r=-0.626$), heptyl acetate (e23; $r=-0.524$), 2-methylpropyl hexanoate (e24; r=−0.599), and hexyl butanoate (e26; $r=-0.629$). However, positive correlations were found between the compounds that belong to the same group. For instance, between alcohols; positive correlation between 1-hexanol (a1) and (E)-2-hexen-1-ol (a4) was found (r=0579), whilst between esters; methyl butanoate (e6) and isopropyl butanoate (e13; $r=0.934$), and also between terpenes. As previously reported in tomato (Zanor et al., 2009), a high positive correlation was found between the terpenes linalool (t6) and alpha-terpineol (t7; $r = 0.965$). This is consistent with that of strawberry study (Zorrilla-Fontanesi et al., 2012) and tomato study (Zanor et al., 2009), a high positive correlation was found between the volatiles that belong to the same group. Since such a strong pair-wise correlations involve volatiles that belong to the same family, a likely explanation is that they are in the same biochemical (biosynthetic) pathway and/or display mutual control by a single enzyme (Zorrilla-Fontanesi et al., 2012).

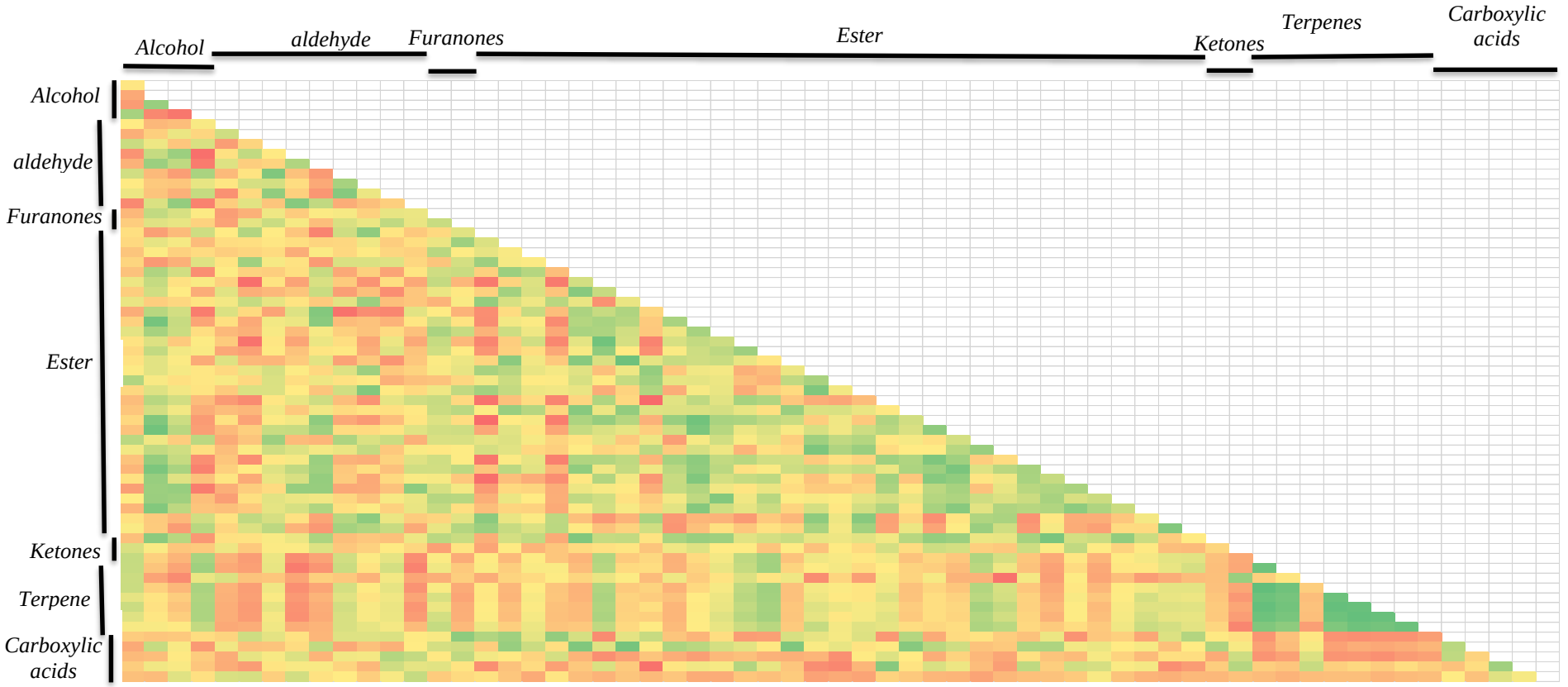


Figure 5.3. Heatmap of correlation matrix (Pearson (n-1)): Table of volatiles compounds. More positive correlations (***r*** > 0.5) are shown in intensifying shades of green. More negative correlations (***r*** < -0.5) are shown in intensifying shades of red. Uncorrelated compounds appear yellow.

The scores and loadings of all nine genotypes for two post-harvest days (day 1 and day 5) are presented in Figure 5.2.b. Four clusters were apparent in the observations plot. The first cluster located on the right side of Figure 5.2.b, containing both samples for RG, RG126, and RG164 as well as one sample of RG100 (day 1). Compounds correlating in this direction include many esters, among them were methyl 2-methylbutanoate (e9 *r*=0.820), 4-methyl-2heptanone (e19 *r*=0.889), heptyl acetate (e23 *r*=0.842), benzaldehyde (a2 *r*=0.822), 1-octanol (a3 *r*=0.596), nonanal (ald5 *r*=0.821), 3-octanone (k1 *r*=0.715), and 2-methylpropanoic acid (c2 *r*=0.587). The second cluster located lower left side of Figure 5.2.b, containing both samples of Hapil and RG169 as well as one sample of RG086 (day 5). Compounds strongly correlated in this position along the principal component in Figure 5.2.a include methyl acetate (e1 *r*=0.702), isopropyl acetate (e4 *r*=0.580), pentanal (ald2 *r*=0.622), (E,E)-2,4-hexadienal (ald7 *r*=0.512), and acetic acid (c1 *r*=0.510).

The third cluster located above left side of the Figure 5.2.b, containing both samples of RG010, one sample of RG086 (day 1) and RG100 (day 5). Four volatiles were correlated to this cluster include ethyl hexanoate (e21 r=0.562), 1-hexanol (a1 *r*=0.355), (E)-2-hexen-1-ol (a4 *r*=0.718), and (E)-2-hexenal (ald6 *r*=0.570). Whilst the final cluster consists solely of one genotype (RG098) for both days, those lie separately as outlier. This particular genotype was chosen because of its low TSS content across shelf life at season 2013 (for more evidence

refer to Chapter 2; section 2.3.3). When compared with Figure 5.2.a, it can be seen that this line in particular was correlated with terpenes: beta-pinene (t1 *r*=0.885), d-limonene (t2 *r*=0.813), beta-ocimene (t4 *r*=0.884), alpha-terpinolene (t5 *r*=0.901), linalool (t6 *r*=0.915), alpha-terpineol (t7 *r*=0.914), and cis-geraniol (t8 *r*=0.811). Overall, four different clusters of the nine genotypes were found based on their volatiles content, however only two of them showed a separation between samples of day 1 and day 5. This may reveal that the genotype influence was stronger than the post-harvest storage time. Previously, Douillard and Guichard (1989) divided 14 frozen strawberry varieties into three clusters based on their volatiles content.

The volatiles compositions of the parental lines were dissimilar (Figure 5.2.b). The first axis mainly discriminated RG-D1 and RG-D5 (below right) samples from H-D1 and H-D5 (below left). This suggests that the parental lines contain relatively different volatile profile, which is consistent with the findings of Zorilla-Fontanesi et al. (2012) where they observed similar separation between the parental lines of their population. This result may be explained by the fact that the parental lines were chosen based on their divergence that were used to generate the mapping population (for more results see Chapter 2; section 2.3.1). However, the volatile compositions of each parent separately over the two shelf life points were very similar and projected close to each other. In addition to the above, the storage effect is shown in Figure 5.2.b and demonstrated only with

RG086 and RG100 samples. The volatile compounds showed also no significant differences while stored at 4 °C between day 1 and day 5, expect for 5 compounds (Table 5.4), which could suggest that the flavour life, defined as the maximum period of storage during which fruit maintained a similar flavour profile (Pelayo et al., 2003), maintained up to day 5. Previously, Pelayo et al. (2003) reported that air-stored strawberries at 5 °C exhibited a flavour life up to 5-9 days depends on cultivar. This suggests that the total area of volatiles was due more to genotype than to post-harvest storage of 5 days.

5.3.2 Sensory attributes

The sensory profile of the nine genotypes of the strawberry population at two different shelf life points (day 1 and day 5) of storage at a commercially relevant temperature of 4 °C was described by a trained panel of experts who, at the end of the profile development, agreed to use 31 terms for the quantitative assessment of the samples. A summary table of sensory attribute scores between the nine genotypes can be found in Table 5.5, including "odour", "taste", "flavour", "mouth sensation", and "aftertaste" attributes. This table shows that 20 out of 31 attributes were found to be significantly different between the nine samples across the two storage points. There was also a highly significant effect of assessor for the majority of the attributes suggesting that the assessors were using the scales differently. However, only 4 attributes had a significant assessor×sample interaction, pointing out that the assessors were ranking the samples in a similar way.

Table 5.5. Mean panel scores for sensory attributes of the nine genotypes of RGxH progeny measured at two different shelf life days.

Code	Attribute	Day	Score[a]									P[b]		
			RG	Hapil	RG010	RG086	RG098	RG100	RG126	RG164	RG169	S	A	I
Odour														
O1	Sweet	1	44.6	50.7	54.8	54.8	48.4	55.6	52.7	48.5	44.3	0.0101	<.0001	NS
		5	50.4	47.3	48.4	45.4	46.2	45.3	53.2	49.7	47.6			
O2	Fermented (lactic acid)	1	8.7	12.6	12.5	11.3	10.5	9.9	13.5	16.4	12.9	NS	<.0001	NS
		5	8.2	12.2	10.6	9.6	7.9	13.4	11.4	11.7	12.5			
O3	Zesty (fresh citrus)	1	14.9^{ab}	16.5^{ab}	13.3^{ab}	14.6^{ab}	18.1^{ab}	12.1^{ab}	13.4^{ab}	13.4^{ab}	13.4^{ab}	0.0228	<.0001	NS
		5	11.8^{b}	15.6^{ab}	19.6^{ab}	15.4^{ab}	16.7^{ab}	19.0^{ab}	16.1^{ab}	15.6^{ab}	21.4^{a}			
O4	Red berry fruit	1	50.2^{ab}	46.1^{ab}	58.5^{a}	50.3^{ab}	47.2^{ab}	54.0^{ab}	50.4^{ab}	49.5^{ab}	46.1^{ab}	0.0308	<.0001	NS
		5	47.6^{ab}	47.4^{ab}	45.5^{ab}	43.1^{b}	45.7^{ab}	43.5^{b}	51.0^{ab}	50.3^{ab}	49.7^{ab}			
O5	Green (green strawberry)	1	18.8^{abc}	18.0^{abc}	**10.1^{c}**	13.5^{abc}	20.4^{abc}	12.2^{abc}	12.2^{abc}	15.8^{abc}	15.3^{abc}	0.0003	<.0001	NS
		5	14.9^{abc}	16.3^{abc}	**23.3^{a}**	20.8^{abc}	18.0^{abc}	10.8^{bc}	14.3^{abc}	14.8^{abc}	21.4^{ab}			
O6	Ripeness (overripe strawberry)	1	39.1	33.9	50.5	50.7	38.9	46.7	52.4	46.0	40.4	<.0001	<.0001	NS
		5	40.0	43.8	35.1	38.5	40.4	41.2	46.9	46.9	40.6			
O7	Rubbery	1	2.9	1.4	1.2	0.7	1.3	0.0	0.3	2.1	1.3	NS	NS	<.0001
		5	1.2	1.7	2.5	0.9	5.6	0.7	0.4	1.8	0.0			

Code	Attribute	Day	Score[a]									P[b]		
			RG	Hapil	RG010	RG086	RG098	RG100	RG126	RG164	RG169	S	A	I
Taste														
T1	Sweet	1	30.9^{d}	39.2^{cd}	52.6^{abc}	46.9^{abc}	54.6^{a}	42.4^{abcd}	53.7^{ab}	43.0^{abcd}	49.2^{abc}	<.0001	<.0001	NS
		5	40.0^{bcd}	43.6^{abcd}	44.6^{abcd}	47.7^{abc}	50.0^{abc}	48.8^{abc}	47.7^{abc}	47.0^{abc}	48.6^{abcd}			
T2	Bitter	1	18.7^{ab}	13.9^{ab}	14.2^{ab}	17.2^{ab}	11.1^{b}	15.4^{ab}	13.1^{ab}	17.8^{ab}	14.2^{ab}	0.0197	<.0001	NS
		5	15.4^{ab}	15.9^{ab}	23.0^{a}	16.9^{ab}	14.9^{ab}	18.7^{ab}	16.8^{ab}	17.5^{ab}	13.1^{b}			
T3	Acidic	1	27.8^{ab}	25.6^{ab}	23.4^{ab}	22.8^{ab}	25.1^{ab}	21.2^{b}	19.6^{b}	27.5^{ab}	26.8^{ab}	0.0281	<.0001	NS
		5	28.2^{ab}	25.2^{ab}	33.0^{a}	22.9^{ab}	25.0^{ab}	28.7^{ab}	25.4^{ab}	25.8^{ab}	28.0^{ab}			
T4	Metallic	1	6.1	4.2	2.7	5.4	3.9	4.7	3.0	4.3	5.4	NS	<.0001	NS
		5	5.5	5.1	5.3	4.6	6.8	5.1	5.2	7.2	5.7			
T5	Savoury	1	7.0	6.0	5.2	4.7	0.9	1.5	3.0	2.6	2.9	NS	<.0001	NS
		5	1.7	2.5	2.7	1.5	1.5	2.1	4.7	2.9	2.2			
Flavour														
F1	Overall strength of flavour	1	44.1^{e}	46.4^{bde}	61.3^{a}	58.2^{a}	59.9^{a}	53.6^{abcde}	55.6^{abcde}	53.7^{abcd}	55.5^{abcd}	<.0001	<.0001	NS
		5	50.3^{abcde}	50.8^{abcde}	57.6^{ab}	53.8^{abcde}	57.6^{abc}	52.0^{abcde}	56.0^{abcd}	55.2^{abcd}	58.7^{a}			
F2	Red berry fruit	1	34.4^{c}	36.6^{bc}	52.2^{a}	48.2^{abc}	53.8^{a}	48.4^{abc}	51.6^{a}	48.0^{abc}	48.9^{abc}	<.0001	<.0001	NS
		5	42.3^{abc}	44.7^{abc}	49.9^{ab}	46.8^{abc}	51.3^{a}	47.3^{abc}	48.9^{abc}	50.3^{ab}	52.4^{ab}			
F3	Green (green strawberry and leafy)	1	21.4	15.2	11.9	14.3	17.3	15.9	12.0	13.0	12.9	NS	<.0001	NS
		5	17.5	15.9	20.2	16.2	12.8	15.6	17.5	15.3	14.1			

Code	Attribute	Day	Score[a]									P[b]		
			RG	Hapil	RG010	RG086	RG098	RG100	RG126	RG164	RG169	S	A	I
F4	Green (kiwi and aromatic)	1	13.5	10.4	11.5	13.2	13.3	10.6	11.3	12.2	13.1	NS	<.0001	NS
		5	10.5	13.2	14.6	11.5	10.3	10.0	10.8	11.0	11.6			
F5	Ripeness	1	30.1^{b}	36.5^{ab}	48.9^{a}	39.5^{ab}	48.1^{a}	44.3^{ab}	50.0^{a}	43.1^{ab}	44.5^{ab}	0.0005	<.0001	NS
		5	33.7^{ab}	40.0^{ab}	39.7^{ab}	44.9^{ab}	46.7^{a}	36.0^{ab}	45.0^{ab}	41.0^{ab}	44.7^{ab}			
F6	Floral (perfume rosey)	1	2.9^{b}	3.8^{b}	9.3^{ab}	8.2^{ab}	6.4^{ab}	5.6^{ab}	13.9^{a}	5.4^{ab}	6.8^{ab}	0.0403	<.0001	0.0144
		5	4.9^{ab}	4.3^{b}	6.9^{ab}	5.8^{ab}	9.2^{ab}	5.0^{ab}	6.9^{ab}	8.6^{ab}	7.5^{ab}			
F7	Cardboard (stale)	1	6.4^{a}	3.5^{ab}	1.1^{ab}	0.6^{ab}	0.4^{b}	1.8^{ab}	2.7^{ab}	1.0^{ab}	1.4^{ab}	0.0340	0.0001	0.0355
		5	2.8^{ab}	2.1^{ab}	2.0^{ab}	0.8^{ab}	0.5^{b}	0.8^{ab}	4.5^{ab}	1.7^{ab}	2.1^{ab}			
F8	Woody	1	0.5	0.9	0.4	0.9	0.9	0.6	0.6	1.9	1.1	NS	<.0001	NS
		5	1.1	1.3	0.6	0.6	0.4	0.9	2.5	2.4	1.8			
Mouth sensation														
M1	Fizzy	1	11.0	7.0	7.1	6.8	5.3	8.6	6.9	8.2	9.8	NS	<.0001	NS
		5	6.9	8.0	8.8	8.7	5.9	9.5	8.0	8.2	9.5			
M2	Mouthdrying	1	20.1	20.4	15.8	18.9	18.9	16.3	17.9	20.9	18.0	0.0164	<.0001	NS
		5	22.3	19.9	24.3	19.6	18.7	20.0	24.2	20.3	23.3			
Aftertaste														
A1	Length of finish	1	35.5	34.3	41.1	38.4	46.2	43.7	45.1	45.2	43.8	0.0186	<.0001	NS
		5	39.5	41.2	46.4	40.6	44.8	39.8	42.0	42.4	44.6			

Code	Attribute	Day	Score[a]									P[b]		
			RG	Hapil	RG010	RG086	RG098	RG100	RG126	RG164	RG169	S	A	I
A2	Acidic	1	23.2^{a}	17.4^{ab}	**12.0^{b}**	14.1^{ab}	19.1^{ab}	16.3^{ab}	16.5^{ab}	20.2^{ab}	16.0^{ab}	0.0019	<.0001	NS
		5	20.9^{ab}	19.7^{ab}	**22.9^{a}**	16.8^{ab}	16.2^{ab}	19.1^{ab}	18.2^{ab}	19.3^{ab}	18.0^{ab}			
A3	Savoury	1	3.3^{ab}	2.1^{ab}	2.3^{ab}	1.1^{ab}	0.7^{b}	1.4^{ab}	1.2^{ab}	0.7^{b}	0.9^{ab}	0.0599	<.0001	NS
		5	1.4^{ab}	1.7^{ab}	1.6^{ab}	1.7^{ab}	1.0^{b}	1.5^{ab}	4.3^{a}	1.7^{ab}	1.9^{ab}			
A4	Cardboard	1	6.7^{a}	3.0^{ab}	2.0^{ab}	1.5^{ab}	0.9^{b}	1.8^{ab}	0.9^{b}	0.5^{b}	1.0^{b}	0.0335	0.0011	NS
		5	1.6^{ab}	0.9^{b}	1.0^{b}	1.3^{ab}	0.3^{b}	0.5^{b}	2.7^{ab}	1.2^{b}	1.6^{ab}			
A5	Metallic	1	9.3^{a}	5.1^{ab}	4.6^{ab}	5.8^{ab}	4.9^{ab}	3.9^{b}	4.3^{ab}	4.9^{ab}	4.8^{ab}	NS	<.0001	NS
		5	6.2^{ab}	5.5^{ab}	5.8^{ab}	5.5^{ab}	6.3^{ab}	6.5^{ab}	5.7^{ab}	7.8^{ab}	5.9^{ab}			
A6	Astringent	1	15.1	11.2	11.9	12.6	12.8	12.7	11.3	18.9	13.0	0.0458	<.0001	NS
		5	15.9	14.8	18.3	13.0	11.7	13.5	15.4	17.4	13.2			
A7	Mouthdrying	1	17.8	18.9	13.3	16.0	16.5	13.9	17.2	17.3	17.8	NS	<.0001	NS
		5	20.6	19.9	21.4	17.3	17.2	19.9	20.2	20.7	19.6			
A8	Salivating	1	14.3^{ab}	8.3^{b}	15.0^{ab}	10.9^{ab}	16.2^{ab}	13.3^{ab}	13.5^{ab}	18.7^{a}	15.2^{ab}	0.0508	<.0001	NS
		5	10.5^{ab}	10.2^{ab}	16.2^{ab}	16.3^{ab}	12.2^{ab}	12.5^{ab}	15.7^{ab}	10.4^{ab}	12.6^{ab}			

[a] Means not labelled with the same letters are significantly different ($p<0.05$); means are from two replicate samples. Those scores significantly different between the two shelf life days are marked in bold. **[b]** Probability, obtained from two-way ANOVA, that there is a difference between means; NS: no significant difference between means ($p<0.05$); S: significance of samples, A: significance of assessors, I: significance of the interaction (S×A).

5.3.2.1 Odour

A summary of seven odour attributes scores can be found in Table 5.5. These attributes include sweet, fermented (lactic acid), zesty (fresh citrus), red berry fruit, green (green strawberry), ripeness (overripe strawberry), and rubbery. Among them, the highest odour scores were for sweet, while the lowest scores were for rubbery. Five attributes (sweet, zesty (fresh citrus), red berry fruit, green (green strawberry), ripeness (overripe strawberry) were significantly different between samples ($P<0.05$). RG010 showed a significant difference between sample of day 1 and day 5 for the green note. However, no other significant differences were found between the two shelf life days.

5.3.2.2 Taste

There were significant differences in three taste attributes out of five between samples. (Table 5.5; $P<0.05$). These attributes include sweet, bitter, and acidic. Sweet taste, the highest taste score, in the parental lines was higher in day 5 comparing to day 1, although it was not significant. This is in alignment with the TSS content, showed earlier in this Chapter (Table 5.1), where both parental lines showed higher TSS content in day 5 comparing to day 1. In addition, three F1 lines (RG086, RG100, and RG164) showed the similar trend to the parents, while other lines (RG010, RG098, RG126, and RG169) showed the opposite (lower values in day 5). A possible explanation for this might be that sweet taste is under

the influence of genotype. Du et al. (2011) reported that sweetness was the second most intense aroma component in two Florida strawberry cultivars.

5.3.2.3 Flavour

Eight flavour attributes scores can be found in Table 5.5. These attributes include overall strength of flavour, red berry fruit, green (green strawberry and leafy), green (kiwi and aromatic), ripeness, floral (perfume rosey), cardboard (stale), and woody. Among them, the highest flavour scores were for overall strength of flavour, while the lowest scores were for woody. Five attributes (overall strength of flavour, red berry fruit, ripeness, floral (perfume rosey), and cardboard (stale)) were significantly different between samples ($P<0.05$).

5.3.2.4 Mouth sensation and aftertaste

Two mouth sensation attributes scores (fizzy and mouthdrying) and eight aftertaste attributes (length of finish, acidic, savoury, cardboard, metallic, astringent, mouthdrying, and salivating) can be found in Table 5.5. For mouth sensation attributes, a significant difference between samples was found for only the mouthdrying attribute ($P<0.05$). For aftertaste attributes, length of finish, acidic, cardboard, and astringent were significantly different between samples ($P<0.05$). RG010 showed a significant difference between sample of day 1 and day 5 for the acidic attribute “aftertaste”. This was expected as this particular line was chosen because of its high content of the TA across shelf life (Selection

protocol for F1 progeny individuals shown in the appendix; section 5.1). However, no other significant differences were found between the two shelf life days.

In conclusion, after 5 days of storage at 4 °C, it is obvious that strawberry fruits of the nine genotypes maintained the acceptable flavour as no attributes showed a statistically significant differences between day 1 and day 5, except RG010 for two attributes (Table 5.5). Changes in strawberry flavour and aroma during storage depend on cultivar, storage condition and duration (Pelayo et al., 2003). Previously, Maul et al. (2000) evaluated the effect of storage temperature on commercially grown tomato flavour and aroma ("Solimar" and "BHN-189"). They found that "BHN-189" tomatoes were significantly lower in ripe aroma, sweetness, and flavour and perceived more sour after 8 days storage at 5 °C, while "Solimar" tomatoes maintained the acceptable flavour up to day 4 (Maul et al., 2000).

5.3.2.5 Principal Component Analysis

Principal components analysis was carried out on the correlation matrix of the nine genotypes at two storage points and all attributes and the first two principal components explained 68 % of the variation in the data (Figure 5.4). The attributes of strawberry taste were mainly contrast of desirable sweet (T1) vs undesirable taste attributes including bitter (T2) and acidic (T3). Sweet taste (T1) were associated with desirable attributes including overall strength of flavour (F1), red berry fruit flavour (F2), and ripeness flavour (F5) (above left). On the other hand,

undesirable taste attributes, bitter (T2) and acidic (T3), were associated with undesirable attributes including zesty odour (O3), green odour (O5), green flavour (F3), acidic aftertaste (A2), and mouthdrying aftertaste (A7). For odour characteristics, desirable attributes including sweet odour (O1), red berry fruit odour (O4), and ripeness odour (O6) were mainly contrast (lower left) vs undesirable sensory attributes including zesty odour (O3) and green odour (O5) (above right). Similarly, Shamaila et al. (1992) reported incongruity between the desirable sensory attributes vs undesirable attributes of strawberry fruit.

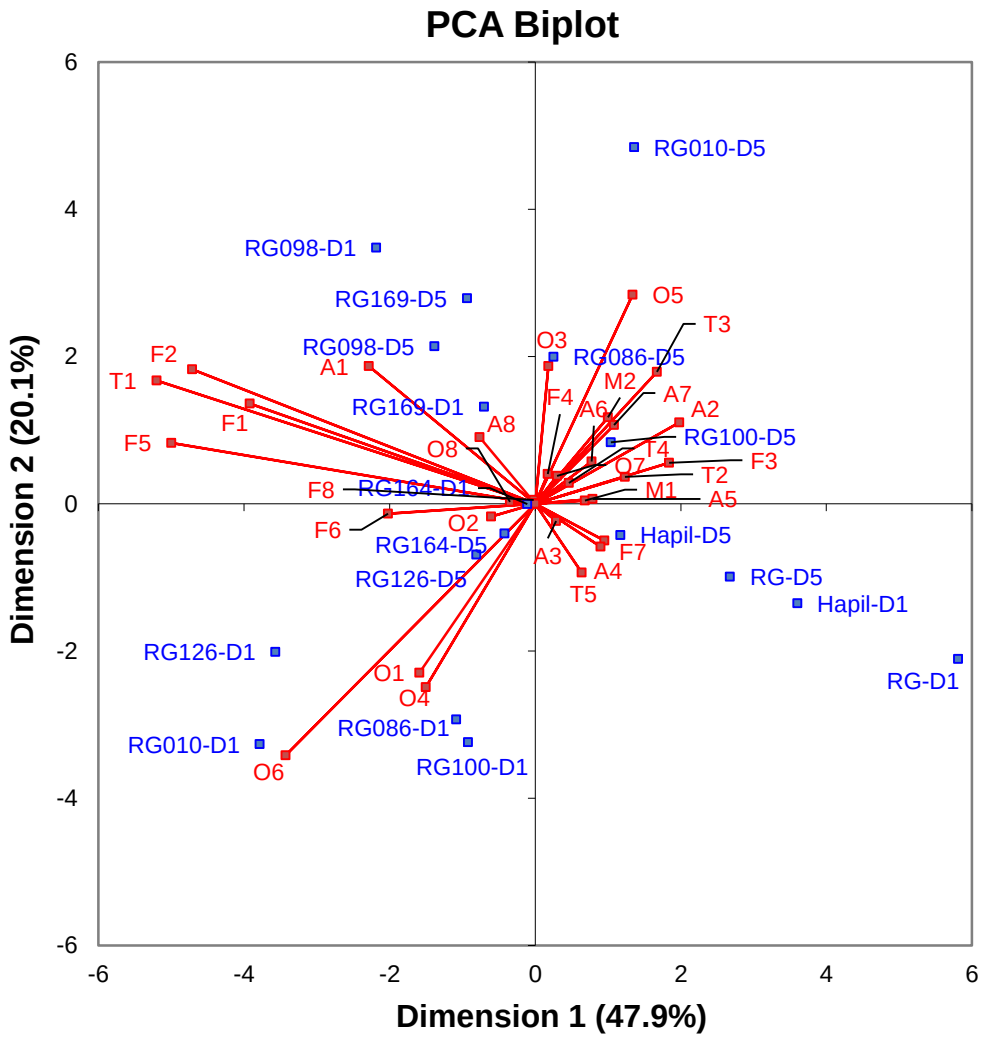


Figure 5.4. Principal component analysis of the nine genotypes of RGxH progeny measured at two different shelf life days showing correlation with sensory attributes (codes refer to compound codes in Table 5.5). Data are means panel scores of two replications (Table 5.5).

The PC1 separated samples of day 1 from day 5 (most of the day 1 samples on the left side and most of the day 5 samples on the right side). Desirable sweet taste (T1), length of finish aftertaste (A1), strength of flavour (F1), red berry fruit flavour (F2), floral flavour (F6), sweet odour (O1), red berry fruit odour (O4), and ripeness odour (O6) were highly correlated with day 1 samples (left side). On the

other hand, zesty odour (O3), green odour (O5), bitter taste (T2), acidic taste (T3), savoury taste (T5), green flavour (F3), cardboard flavour (F7), acidic aftertaste (A2), and mouthdrying aftertaste (A7) were correlated with the day 5 samples (right side).

The parental lines samples for day 1 and 5 were clustered on PC1 or PC2 (lower right). However, the PC1 separating day 1 samples from day 5 samples for RG010, RG086, and RG100, while PC2 separating day 1 sample from day 5 sample for RG164. Sweet odour (O1), red berry fruit odour (O4), and ripeness odour (O6) were associated with the day 1 samples, whereas zesty odour (O3), green odour (O5), acidic taste (T3), metallic taste (T4), green flavour (F3), mouthdrying sensation (M2), acidic aftertaste (A2), and mouthdrying aftertaste (A7) were associated with the day 5 samples. Interestingly, PCA for volatiles also separated day 1 samples from day 5 samples for RG086 and RG100 (Figure 5.3), which could suggest the correlation between volatiles and sensory perception.

5.3.3 Relating sensory to instrumental data

Principal component analysis was performed to summarise the differences and relationships among the sensory and instrumental data in the nine genotypes at two different post-harvest days (day 1 and day 5). The first three PCs (PC1, PC2 and PC3) accounted for 59.56 % of the variation in the data and were presented in Figure 5.6. The majority of explained variation was found in the PC1 (32.34 %), which mainly separates most of the sensory attributes (taste, flavour, moth

sensation and aftertaste), seven volatiles (mainly esters), and two physiological traits (TSS and TA). It separates the desirable sensory traits from the undesirable traits. PC2 (17.07 %) mainly separates three odour attributes (O3, O4 and O5), few volatiles (a3, a4, ald4), as well as three physiological traits (pelargonidin 'Pel', TSS, a* value and b* value). Moreover, the PC3 (10.15 %) identifies a dimension characterised by undesirable attributes include fermented odour (O2), off-flavour (O8), woody flavour (F8) and astringent aftertaste (A6) as well as two volatiles (e10 and ald5), and ellagic acid content 'EA'. Significant correlations (Pearson n-1) between phytochemicals and sensory attributes were also summarised in Figure 5.5, and the regressed factor loadings of each variable were presented in supplementary data (Full table shown in Appendix; section 5.4).

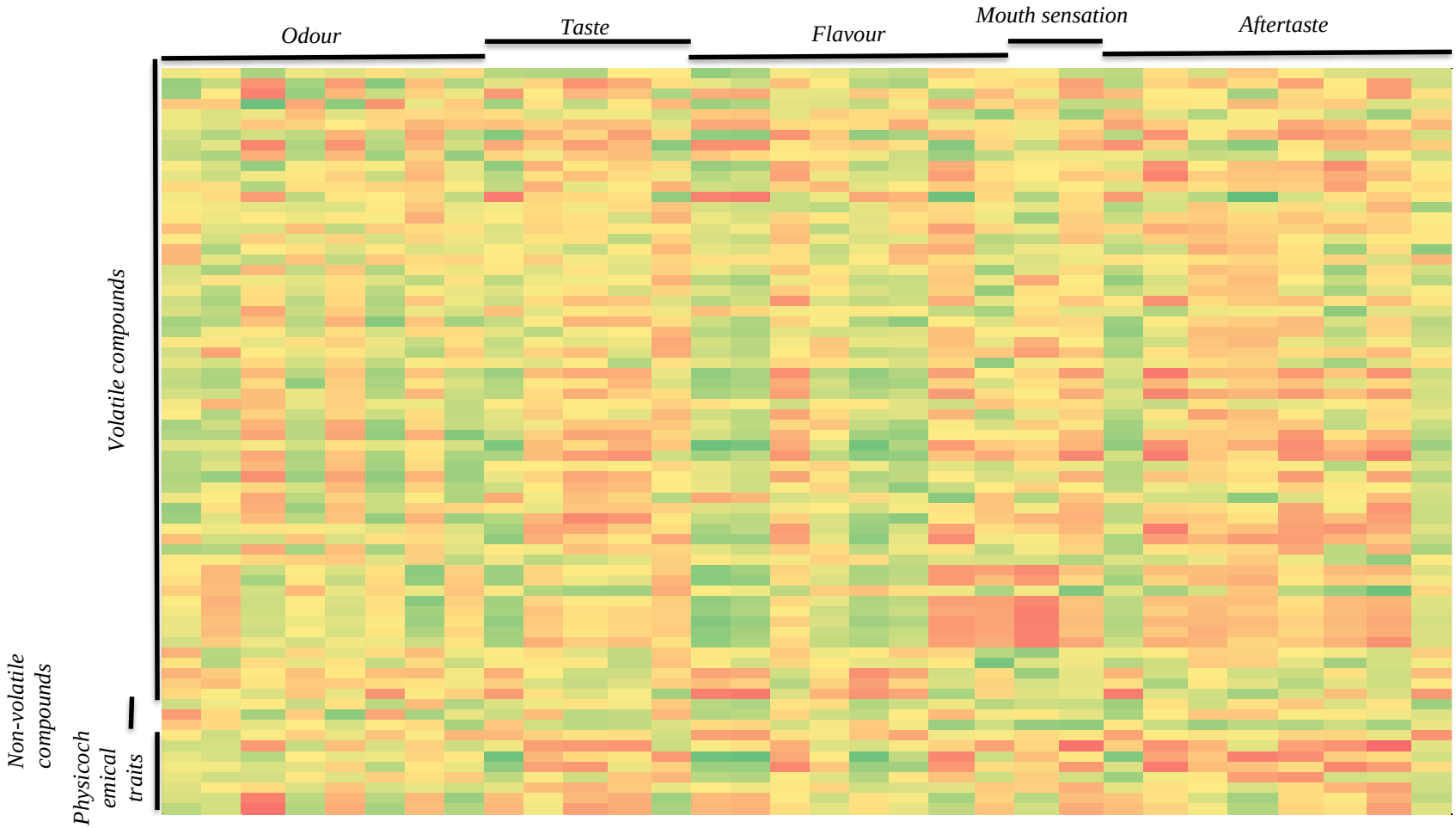


Figure 5.5. Heatmap of correlation matrix (Pearson (n-1)): Table of combined data (sensory and physicochemical date). More positive correlations (***r*** > 0.5) are shown in intensifying shades of green. More negative correlations (***r*** < -0.5) are shown in intensifying shades of red. Uncorrelated compounds appear yellow**.**

PC1 vs. PC2: Although many volatile compounds were detected in strawberry fruits of nine lines (Figure 5.6.a), only a few of them significantly contribute to the flavour character. Therefore, the mixture of the sensory analysis with the instrumental analysis will provide better insights into the impact of volatile compounds on fruit flavour than either alone (Song and Forney, 2008). The relative distribution of these compounds with sensory attributes is presented in the PCA biplots (Figure 5.6.a and 5.6.b). Based on the sensory data discussed above (5.3.2.5), sweet taste (T1), overall strength of flavour (F1), red berry fruit flavour (F2), and ripeness flavour (F5) are the desirable sensory attributes. These attributes were mostly correlated with three volatile groups; esters, terpenes, and aldehyde; however, other volatile groups were partially correlated (Figure 5.5). For example, the overall strength of flavour (F1) was the most sensory attributes that has high positive-negative correlations with the physicochemical data ($P<0.01$). It has high positive correlations with 1-hexanol (a1), hexanal (ald3), (E)-2-hexenal (ald6), 3-methylbutyl acetate (e16), hexyl acetate (e22), all eight terpenes (except eucalyptol), and TSS. It was also negatively correlated with pentanal (ald2), (Z)-2-decenal (ald9), and acetic acid (c1). Previously, Jouquand et al. (2008) reported that esters, terpenes, aldehyde and furanones were reported as the major aroma compounds in strawberry. What was surprising that hexanal (ald3) and (E)-2-hexenal (ald6), those known for their green note (Jetti et al., 2007), were positively correlated with the desirable characteristics such as the overall strength of flavour (F1) and sweet taste (T1). However, this inconsistency

may be due to fact that sensory impact of some volatile compounds may be masked or improved by other volatiles (Grosch, 2001; McBride, 1990). It was found by Schwieterman et al. (2014) that sweet intensity was the strongest driver of overall liking of strawberry. Another possible explanation is that the fruity note generated by ester compounds was stronger than the green note generated by aldehyde compounds.

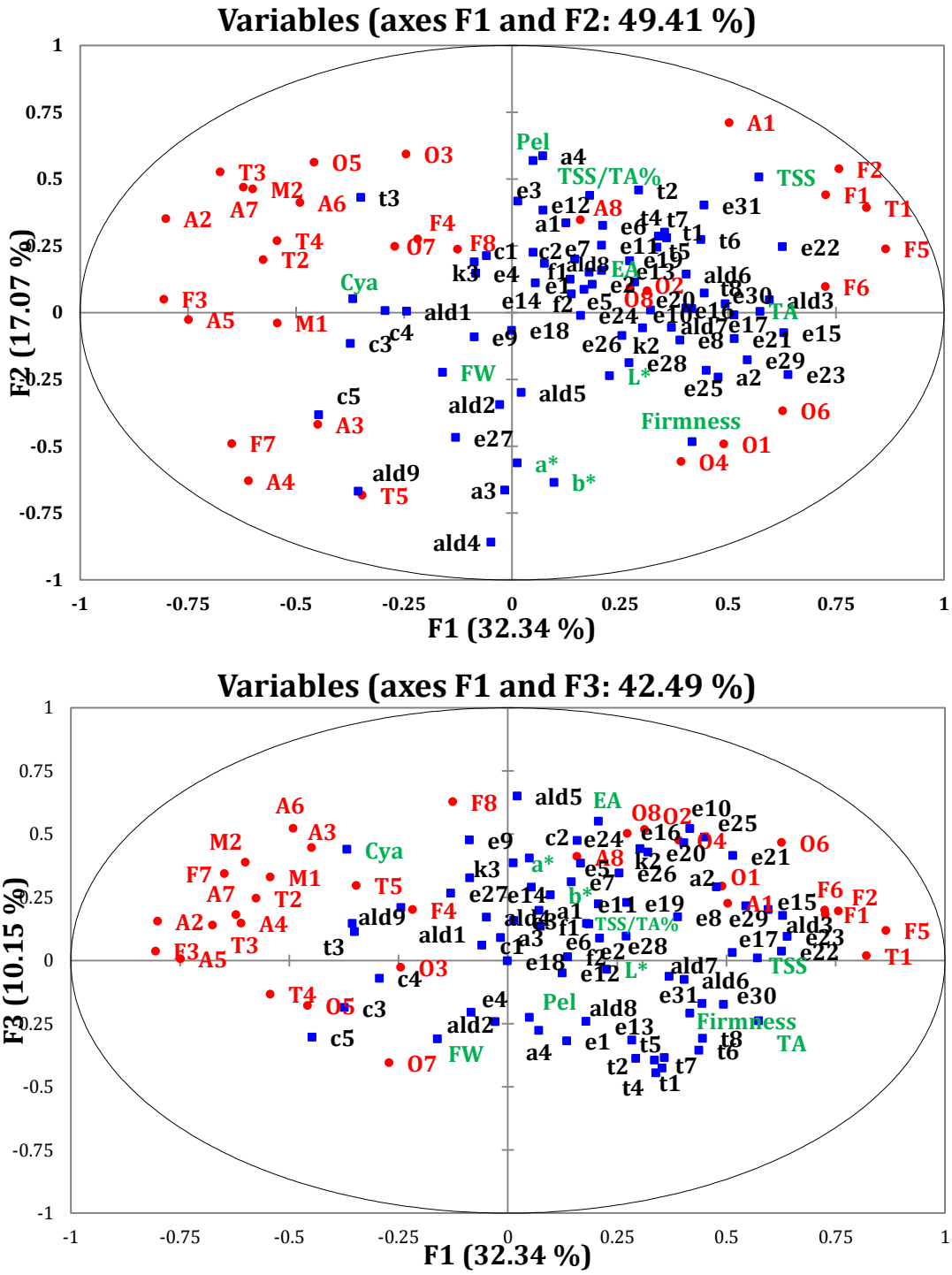


Figure 5.6. Principal component analysis of the nine genotypes of RGxH progeny measured at two different shelf life days showing distribution of variables (● = sensory variables (red), ■ = instrumental variables (black is volatiles and green is physiological traits), codes on table refer to compound codes in Table 5.3 and 5.5). Pel is pelargonidin, Cya is cyanidin, and EA is ellagic acid. Data are mean values of sensory panel scores (Table 5.5), physiological traits (Table 5.1), non-volatile compounds (Table 5.2), and volatile compounds (5.4).

Interestingly, sweet taste (T1) and overall strength of flavour (F1) were significantly highly correlated with TSS (P<0.01; r=0.722 and r=0.733), revealing that sugar content contributes to the perception of fruit flavour (taste) and the higher the content, the sweeter the fruit (Figure 5.6). Previously, Schwieterman et al. (2014) found that sweetness intensity was the strongest driver of overall liking measured in strawberry. Resende et al. (2008) also reported a significantly positive correlation between TSS and strawberry flavour (r=0.98). On the other hand, ethyl acetate (e3), which was previously reported as a fermentative metabolite in strawberry (Hakala et al., 2002; Pelayo-Zaldivar et al., 2007), was positively correlated (P<0.01) with fermented odour (O2) and off-flavour odour (O8), however it was not highly correlated. Additionally, pelargonidin content was correlated negatively to all colour parameters (L*; r=−0.186, a*; r=−0.486, and b*; r=−0.613; Figure 5.6). Here one has to be careful because a high content of pelargonidin means low scores of L*, a* and b* values which indicates more darkness. This confirms the relationship between anthocyanin and fruit colour (for more results refer to Chapter 3; section 3.3.3.3).

Again, undesirable sensory attributes (bitter taste (T2), acidic taste (T3), zesty odour (O3), green odour (O5), green flavour (F3), acidic aftertaste (A2), and mouthdrying aftertaste (A7)) clustered along the negative side of PC1 (above left; Figure 5.6.a). This was similar to the sensory data discussed above (5.3.2.5). These attributes correlated with one terpene compound (t3; eucalyptol) that has

highly negative correlation with TSS (r=−0.887) and was not clustered within all terpenes on the PCA of volatiles (Figure 5.3). This compound was reported to impart a pine note in muskmelon and was higher in the acidic verities (Lignou et al., 2013), which explain the acidic perception associated with day 5 samples correlated to the undesirable sensory attributes (Figure 5.6.a and 5.6.b).

Six genotypes out of nine showed a separation between day 1 and day 5 samples (Hapil, RG010, RG086, RG100, RG126 and RG164). Three of these six genotypes also showed a separation between day 1 and day 5 samples for volatiles (RG086 and RG100; Figure 5.3) and sensory attributes (RG010, RG086 and RG100; Figure 5.4) which may explain their separation in Figure 5.6 and 5.7. Interestingly, RG010-D5 lies separately from the other genotypes (above left). This particular genotype, which was chosen for its high TA content across shelf life in season 2013, outlies also by sensory perception (Figure 5.4). It was the sole genotype to show significant differences across shelf life for two sensory attributes (green odour (O5) and acidic aftertaste (A2); Table 5.5) which may explain its position herein as an outlier.

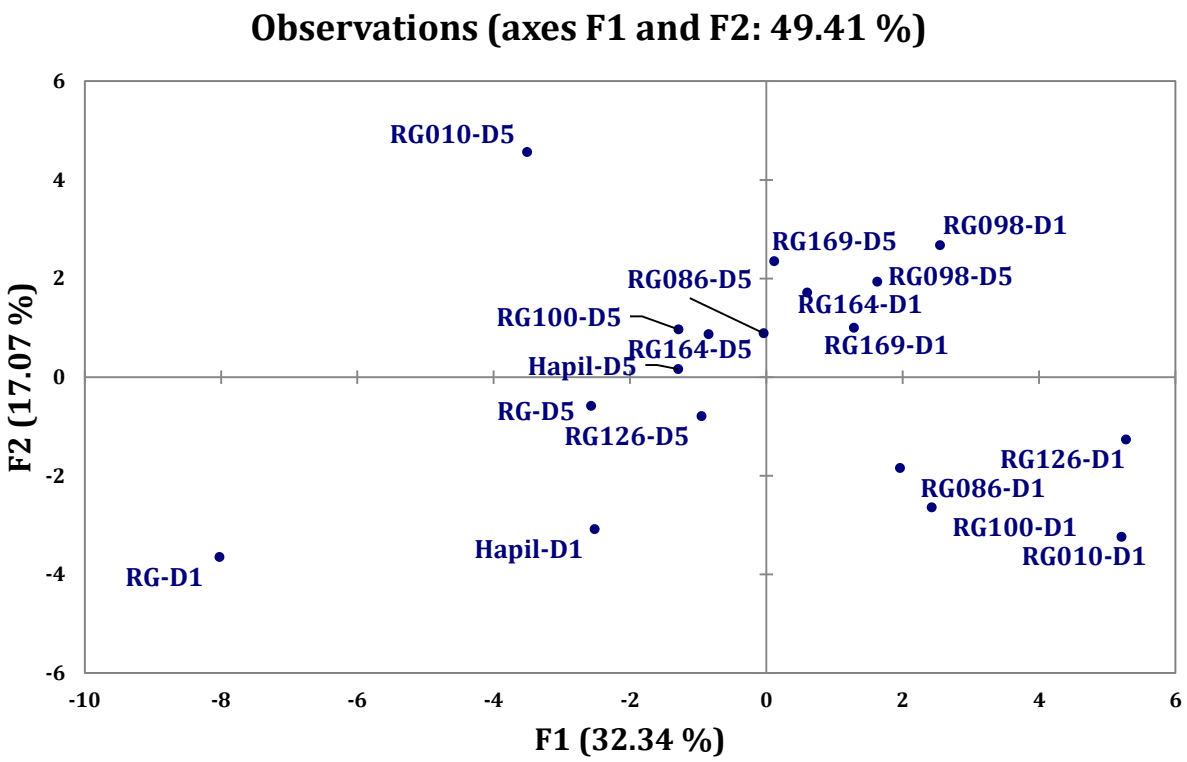
Firmness was correlated positively along the PC1 (Factor loading=0.417; Figure 5.5) with some desirable sensory traits include sweet odour (O1), red berry fruit odour (O4) and ripeness odour (O6), however these were not high correlations (r=0.188-0.276). Interestingly, all these traits were correlated nicely with day 1 samples for RG010, RG086, RG100, and RG126. On the other hand, firmness

was negatively highly correlated with zesty odour (O3; r=−0.489), acidic taste (T3; r=−0.494), metallic taste (T4; r=−0.523), mouthdrying (M2; r=−0.722), acidic aftertaste (A2; r=−0.535), astringent aftertaste (A6; r=−0.534) and mouthdrying aftertaste (A7; r=−0.784) as well as eucalyptol (t3). This indicates that zesty odour, which described the fresh citrus odour of the fresh fruits, decrease with decreasing firmness (increase softening). It also indicates that acidic taste (T3) and acidic aftertaste (A2) increase with decreasing firmness (increase softening). Moreover, the highest negative correlation was found between mouthdrying (M2 and A7) and firmness. A possible explanation for this is that as strawberries are very susceptible to water loss that leads to several consequences, one of which is fresh weight reduction. Thus, fruits at day 5 (less firm) contain less water, which make the flavour perception of these fruits drier.

The most abundant esters identified were ethyl propanoate (both days), methyl 2-methylbutanoate (day 1), pentyl acetate (day 1), 2-methylpropyl butanoate (both days), ethyl hexanoate (both days), and (E)-2-hexen-1-ol acetate (both days). Among them, (E)-2-hexen-1-ol acetate has some positive correlations with some desirable attributes including sweet taste (P<0.01; T1 r=0.555), overall strength of flavour (P<0.01; F1 r=0.482), red berry odour (P<0.01; F2 r=0.501), and ripeness odour (P<0.01; F5 r=0.575) as well as a negative correlation with undesirable attributes such as cardboard flavour (P<0.01; F7 r=−0.568) (Figure 5.5 & Figure 5.6. Similarly, a terpene compound (linalool), the common fruit

volatile with a floral/rose odour, has some positive correlations with some desirable attributes including sweet taste (P<0.01; T1 r=0.536), overall strength of flavour (P<0.01; F1 r=0.636) and red berry odour (P<0.01; F2 r=0.515), as well as some negative correlations with undesirable attributes such as cardboard flavour (P<0.01; F7 r=−0.501), and fizzy mouth sensation (P<0.01; M1 r=−0.636).

PC1 vs. PC3: The PC3 accounts for 10.15 % of the explained variation (Figure 5.6.b). It identifies a dimension characterised by undesirable attributes including fermented odour (O2), off-flavour (O8), woody flavour (F8) and astringent aftertaste (A6) as well as two volatiles (e10 and ald5), and ellagic acid content 'EA'. However, other characteristics, those were associated with PC1 and PC2, are still clustered in the same way. The desirable sensory attributes clustered along the positive side of PC1, while the undesirable on the negative side (Figure 5.6.b). Interestingly, sweet odour (O10), red berry odour (O4) and ripeness odour (O6) joined nicely the other desirable characteristics on the positive side of PC1, those were associated nicely with sugar content (TSS) as well as mostly with day 1 samples as shown in Figure 5.7.b.



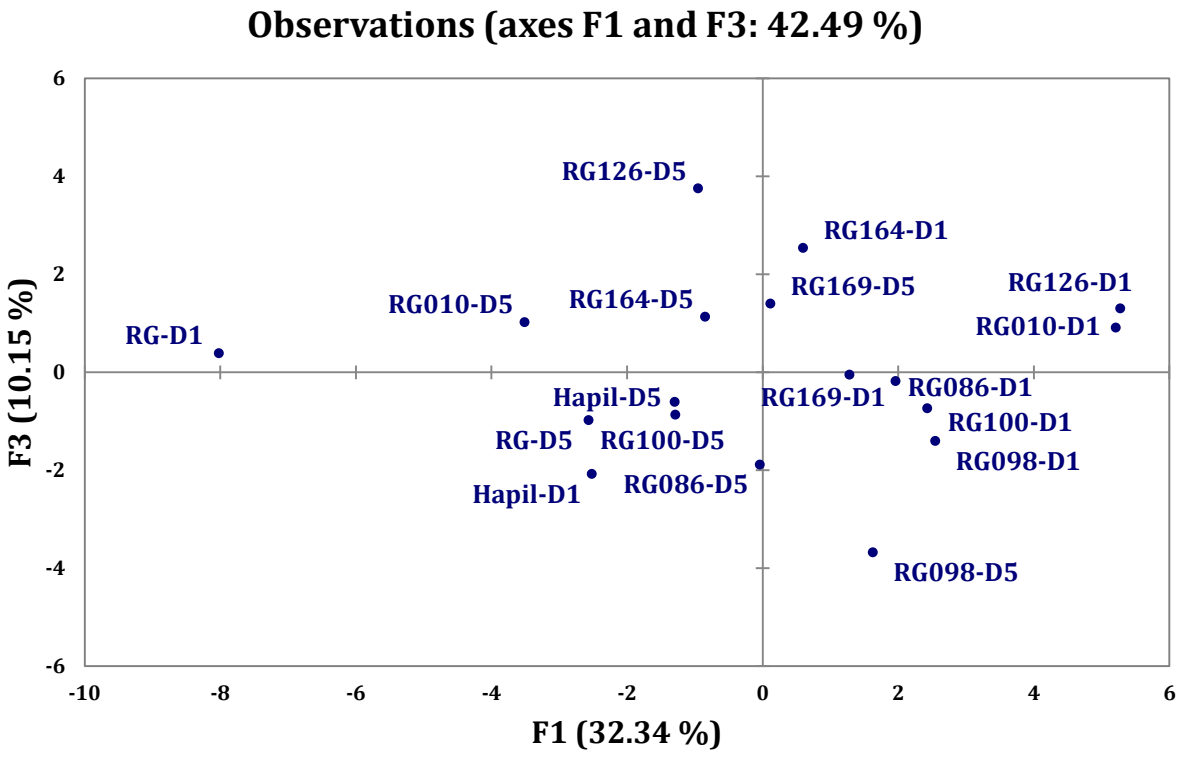


Figure 5.7. Principal component analysis of the nine genotypes of RGxH progeny measured at two different shelf life days showing distribution of samples.

5.4 **Summary**

Both sensory and instrumental analysis of physiochemical traits, volatile and non-volatile compounds have identified significant differences between the nine lines of strawberry samples that can be attributed to either the genotype or the shelf life. For FW and firmness, all nine genotypes decreased with storage, however, for TSS, TA, TSS/TA ratio and phenolic content, the genetic variation was the dominant factor. Among the detected volatile compounds, the most abundant compounds in terms of the number of detected compounds and quantities on both days were esters (31 compounds), followed by aldehydes (9 compounds) and terpene derivatives (8 compounds). The results presented confirm the role of volatile compounds (mainly esters, terpenes and aldehydes) along with some physical traits (mainly TSS, TA and their ratio) in sensory perception.

Samples of day 1 exhibited the highest amount of esters and terpenes, those samples were generally described by the assessors as having desirable sensory attributes. Among them, (E)-2-hexen-1-ol acetate (ester; contribute to the fruity and floral notes) and furaneol (furanone; contribute to the sweet note), exhibited higher levels at day 1, have a positive correlation with some desirable attributes including sweet taste, overall strength of flavour, red berry odour, and ripeness odour. In addition, a significantly positive correlation was found between sweet taste and sugar content (TSS) revealing that sugar content contributes to the perception of fruit flavour. However, the combination of low TSS/TA ratio in

samples of day 5, along with other compounds, like eucalyptol (terpene; contribute to the pine note and had highly negative correlation with TSS) resulted in a fruit with an acidic perception. Overall, samples of day 1 were mostly correlated with the desirable attributes while day 5 samples mostly correlated with the undesirable attributes. This study suggests that shelf life variation is substantial in TSS, TA and their ratio, firmness and sensory characteristics such as sweet taste, overall strength of flavour, red berry odour, and ripeness odour.

Chapter 6 : General discussion

Over the last few years, strawberries have seen increased demand in the market (Hummer and Hancock, 2009; Zorrilla-Fontanesi et al., 2011) due to their freshness, exceptional flavour and health benefits (Ayala-Zavala et al., 2004; Halbwirth et al., 2006). Strawberries are known for their richness in vitamins, minerals, anthocyanin, flavonoids, and phenolic acids, but are also highly perishable. Thus, breeding new strawberry cultivars with improved nutritional and quality traits is an important goal for breeding programmes in terms of the sustainability and competitiveness of strawberry production.

Identifying quantitative trait loci (QTL) for strawberry traits could lead to a better understanding of the associations between strawberry phenotypes and their genotype, how quality is regulated at the genetic level and how various traits are genetically correlated, thus facilitating molecular marker development. Therefore, the primary aim of this study was to map the variation in quality traits in a segregating F1 strawberry population progeny and provide new data to assist breeding programmes in developing cultivars with improved fruit quality traits.

To date, QTL studies of fruit quality traits have focused only on those traits measured at harvest, while the majority of the fruit typically reaches the consumer after a period of several days in cold post-harvest storage. Therefore, two studies were conducted over different strawberry shelf life storage periods using an F1 progeny (*Fragaria x ananassa Duch.*), which is derived from the cross of

Redgauntlet x Hapil (RGxH) strawberry cultivars. The two major goals of these studies were:

I. To identify the QTL linked to the traits of interest over different shelf life lengths using a SNP-based genetic linkage map;
II. To evaluate the flavour profiles of seven genotypes of the RGxH F1 strawberry population and their parental lines at two shelf life storage points at the commercially standard storage temperature of 4 °C.

This has provided data at the phenotypic and genetic level that has contributed to a better understanding of the associations between strawberry phenotypes and their genotype. This research will contribute to the improvement of strawberry breeding processes by reducing time and costs through the application of a marker-assisted selection approach (MAS).

6.1 Mapping QTL underlying fruit quality traits

The aim of this study was to address the influence of genotype, shelf life storage, and cultivation site on strawberry quality traits and detect the QTL linked to these traits. Data were collected over three post-harvest days for two sequential seasons in different locations. Due to practical constraints, only 20 progeny lines were cultivated over the two seasons. However, these 20 lines showed varying phenotypic performance over the two seasons/environments in which the environmental effect was significant ($P < 0.05$). Thus, as it was not possible to

map the combined data set of all the traits/genotypes together, the data were treated as two distinct datasets.

In the two experiments over the sequential seasons (2013 and 2014), 51 post-harvest traits of the strawberry mapping population were phenotyped (days 1 and 7 for 2013 and days 1, 4 and 7 for 2014). Among the associated parental lines used, the most noteworthy difference was found in their anthocyanin content, or main colour compounds. This finding supports the fact that the content of phenolic compounds (including phenolic acid and anthocyanin) in strawberries can vary between cultivars (Aaby et al., 2012; Crespo et al., 2010).

Environmental effects, including those for cultivation site, practice and conditions, were seen in the post-harvest quality trait results ($P < 0.05$). Such environmental effects may influence genetic variability and therefore may have reduced the number of significant overlapping QTL between both experiments. One exception was fruit lightness (L* value), which did not show significant variation between the two sites, which is advantageous for breeding programs that use MAS. Due to the significant environmental effect found that was linked to fruit quality traits, further validation of the detected QTL over different cultivation sites using the same set of lines across both sites is necessary in future to evaluate the effect of cultivation site on fruit quality, and therefore to assess the GxE impact on the associated QTL for these quality traits.

To map the QTL associated with shelf life variation and nutritional quality traits, the first high-throughput genotyping array for octoploid *Fragaria*, the Affymetrix IStraw90® Axiom array, as described by Bassil et al. (2015), was used by EMR (New Road, East Malling, Kent) for genotyping the RGxH mapping progeny of 140 individuals. This map contains a total of 3933 SNPs distributed over 28 linkage groups and covers a total length of 2,624.7 cM. However, due to the limited computational power of the MapQTL application, the number of SNPs used had to be reduced to 523, with as average interval of 5 cM between markers, as the software could not process the entire data set.

As a result, 47 QTL (8 QTL for season 2013 and 39 QTL for season 2014) over 22 linkage groups were identified, with an average explained variance of 18.8% and 19.9% for seasons 2013 and 2014, respectively. In season 2013, three major QTL were detected that accounted for more than 20% of the explained population variance for fruit lightness (L* value) and TSS/TA ratio, whereas 17 out of the 39 major QTL were detected in season 2014. Of the 17 major QTL detected in season 2014, three major QTL accounted for >30 % of phenotypic variance. These QTL related to FW-4-14 (LG3A), FW-7-14 (LG3A) and TSS-7-14 (LG5A), with explained variances of 37.5%, 37.8% and 38.2%, respectively. These results suggest that these QTL have gene(s) that could control the quantitative character of these traits. However, further validation of these markers in a larger strawberry

germplasm collection would be necessary to confirm the significance levels of the markers detected in these results.

The number of detected QTL per trait ranged between one QTL (TSS/TA-1-13, a-7-13, Pel-1-14, Firmness-4-14, L-4-14, a-4-14, TSS-4-14, and TA-4-14) and five QTL (TSS-7-14 and TSS/TA-1-14). The number of QTL that affected each individual trait could suggest the complexity of the biological processes and metabolic pathways underlying these traits (Lerceteau-Kohler et al., 2012). QTL for fruit quality traits for various shelf life points were also detected for 7 out of 11 traits, including FW, fruit lightness (L* value), TSS, TA, TSS/TA ratio, ellagic acid, and pelargonidin. However, only QTL linked with FW and TSS/TA ratios were co-located on the same LG over shelf life points, suggesting a possible pleiotropic effect.

The 2014 season results showed that three QTLs linked with FW were co-located for all three shelf life points, suggesting that this is a major QTL controlling FW which can be used in strawberry breeding programs that are aimed at the production of improved varieties with overall fruit quality traits, including FW. However, validation analysis of the markers would be necessary to determine which of these markers are reliable in enhancing breeding efficiency through marker-assisted breeding (MAB). In addition, two QTL linked with TSS/TA ratio were also detected in the same LG over shelf life points in season 2014. Such results indicate that the same gene(s) likely dictate the variation of these two traits,

regardless of shelf life. In contrast, five QTL for TSS-7-14 and TSS/TA-1-14 were detected for different LGs, which suggests that many independent loci of small effects control these traits.

Broad-sense heritability "H^2", including all forms of genetic heritability (additive, dominance, and epistatic variation), can also affect phenotypic variation in the population and the genetic control of QTL. This study demonstrated that high heritability values were observed ($H^2 > 0.5$) for 18 out of 51 traits, suggesting that genetic factors contributed more than environmental factors. Furthermore, 12 of the 18 analysed traits, including TSS, TA, TSS/TA ratio, and phenolic compounds (ellagic acid and pelargonidin), showed very high H^2 values ($H^2 > 0.7$). Such values may indicate that these phytochemicals are less affected by environmental conditions, or that these phytochemicals are strongly controlled by genetic factors, which by definition are inherited from parent organisms.

Although an F1 heterozygous population with a low number of overlapping lines between the two seasons was used here, co-locations were observed for some QTL for closely correlated quality traits, as discussed in Chapter 4 (Section 4.3.2). Co-location of QTL was detected for pelargonidin content (Pel-4-14, with Hapil allele positive contribution) and value for lightness (L-7-13, with RG positive allele positive contribution) at LG2B, which is the only co-location identified for different years/conditions, suggesting an antagonistic pleiotropic effect. This was in agreement with the negative correlations found between L* values and

pelargonidin content (Pearson's correlation; $p \leq 0.01$), which may explain the commonalities in the genetic regulation between anthocyanin content and the redness of strawberry fruit. Co-localization was also detected for Cya-1-14 and TSS-4-14 (Pearson's correlation; $p \leq 0.01$) at LG1A, both with the Hapil allele positive contributing to higher trait values, suggesting a pleiotropic effect at this particular LG (El-Soda et al., 2014). In addition, it is known that sugars are the initial precursor of the anthocyanin biosynthesis, which may explain the correlation between these two traits (Hrazdina et al., 1984; Ruhnan and Forkmann, 1988; Teusch et al., 1987). Further phenotyping and validation analysis of these traits could provide important findings about the possibility of combining these two traits in strawberry breeding.

The results of this study provide novel information on the genetic architecture of fruit quality traits across shelf life points that are relevant for strawberry breeding. The SNP markers identified here that linked to QTL for the traits of interest constitute a first step toward improving strawberry MAS programmes. Furthermore, the highly heritable traits and the number of major QTL identified in this study suggest consistent associations between phenotypes and genotype. However, further testing would be necessary to confirm the significance and stability of the identified QTL in other mapping of octoploid strawberry populations in different environments and over several years before they are considered in breeding programmes for MAS (Kenis et al., 2008).

In addition, the results revealed a number of QTL that control the quality traits over shelf life storage in the *Fragaria x ananassa* strawberry, which suggests the potential to improve these traits of interest. Furthermore, the findings support the notion that the plant characteristics and fruit quality traits of the octoploid strawberry are complex, and that a large number of genes may control each single trait. However, as many QTL were co-located in this study, breeding programmes should take care when applying these results. To this point, a number of potential study limitations were identified during the QTL analysis, including:

- As a result of the computational limitations of the MapQTL software in processing the marker overload, the number of SNPs was reduced from 3933 to 523, and was distributed over the 28 LGs.
- The LOD output generated by the permutation test was relatively high for all traits. Therefore, based on previous recommendations (Van Ooijen, 1999), a LOD threshold of 3.2 was used to identify potential QTL.
- A shortage in the number of genotypes for the two seasons can be attributed to the following:
    - Lack of fieldwork experience at the beginning of the study;
    - The number of the quality traits measured. In future work, it would be better to focus on a fewer number of traits in order to cover as many genetic lines as possible and use the same set of lines across seasons.

6.2 Sensory, volatile and physicochemical analysis of nine genotypes of the strawberry population

This experiment evaluated the flavour profiles of seven genotypes of the RGxH F1 strawberry population and their parental lines in order to detect correlations between sensory and instrumental data. These genotypes were selected based on their sugar and acid content, and were monitored at two shelf life points (days 1 and 5) at a commercially relevant storage temperature of 4 °C. A total of 61 compounds were identified for the nine genotypes at the two shelf life points. For both days, esters were found to be the most abundant compounds (31 compounds), followed by aldehydes (9 compounds) and terpenes (8 compounds).

Ten trained sensory panellists rated strawberry puree samples stored at 4 °C taken from storage days 1 and 5. Thirty sensory attributes were evaluated, including odour, taste, flavour, mouth sensation and aftertaste. For odour and taste attributes, sweet (T1 and O1) showed the highest score, and among flavour attributes overall strength of flavour (F1) was the highest. A PCA on sensory analysis showed a clear separation between desirable attributes (T1, F1, F2, F5, O1, O4 and O6) and undesirable attributes (T2, T3, O3, O5, F3, A2 and A7). Furthermore, these desirable attributes correlated with most day 1 samples, while the undesirable attributes correlated with most samples from day 5. In addition, the results support the role of strawberry storage on flavour perception, including factors related to volatile compound content (mainly esters, terpenes and

aldehydes) and some sensory attributes. In addition, a significantly positive correlation was found between sweetness (T1) and sugar content (TSS), which suggests that sugar content contributes to the perception of fruit flavour.

Samples collected on day 1 exhibited the highest amount of esters and terpenes, and assessors generally described them as having desirable sensory attributes. Among them, (E)-2-hexen-1-ol acetate (ester, which contributes to fruity and floral notes) and furaneol (furanone, which contributes to the sweet note), exhibited higher levels for the day 1 samples, and correlated positively with some desirable attributes, including sweet taste (T1), overall strength of flavour (F1), red berry odour (O4), and ripeness odour (O6). On the other hand, for the day 5 samples, the combination of a low TSS/TA ratio along with other compounds, such as eucalyptol (terpene, which contributes to the pine note and was highly negatively correlated with TSS) resulted in a fruit with an acidic perception. Furthermore, three out of seven offspring lines (RG010, RG086 and RG100) showed a separation in the PCA plot between days 1 and 5. Two out of these three lines (RG010 and RG086) were also separated by their volatile content, suggesting the influence of volatile compounds on sensory perception.

In sum, the study results showed correlations between sensory attributes, volatile compounds and physicochemical data. Furthermore, these results confirm the role volatile compounds (mainly esters, terpenes and aldehydes) and some physical traits (mainly TSS, TA and their ratio) can play in sensory perception.

6.3 **Future Work**

6.3.1 Mapping QTL underlying fruit quality traits

These findings represent a starting point and could facilitate improvements in further work in strawberry, as they indicate the most likely candidate regions that may influence polyphenol production and other important traits over shelf life points. Thus, these results could contribute to future studies, including those associated with molecular markers and their underlying genes, which could then be used to drive marker-assisted selection (MAS) processes in developing superior strawberry cultivars with greater nutritional and quality traits.

Fine-mapping and QTL validation

The availability of novel high density SNP-based linkage map suggests areas for future research, as it remains necessary to test the stability of the identified major QTL resulting from the current study in other octoploid strawberry populations in different environments and over time before candidate genes can be identified using the fine-mapping approach, which is of interest to breeders. The benefits of the fine-mapping approach could help to simplify the validation of the identified QTL within the current population and confirm the genes underlying these QTL, making breeding for traits of interest more reliable and effective. Furthermore, this study could also be useful in determining candidate genes associated with some major QTL, first in parental lines, and then, if significant differences are

found, in the F1 lines to map expression QTL, to see if they co-locate to the QTL identified in this study.

Developing a robust QTL mapping programme

Another approach to providing more data lies in developing a more robust QTL mapping programme that can process the dense SNP-based linkage map containing 3933 unique SNPs, which could assist in the fine mapping of detected QTL. This research conducted studies to refine QTL position through the saturation of the regions under significant QTL with as many markers as possible. However, for this research, due to computational limitations of the MapQTL software in processing the marker overload (see Section 2.5.5.1), the number of SNPs was reduced to 523, and was distributed over the 28 LGs. Nine major QTL were in question for refined positions, and the results of this refinement are summarized in Section 4.3.4, Table 4.8. Six of the nine major QTL remained the same after adding 1 cM intervals to the QTL positions, which suggests their true association with the SNPs at the 5 cM interval. It would be worth developing a QTL mapping programme that can process dense SNP-based linkage maps to realize the full benefit of the available SNP-based map and expand fine-mapping analysis.

6.3.2 Sensory, volatile and physicochemical analysis of nine genotypes of the strawberry population

Regarding flavour profiles, statistical analysis of the sensory, volatile and physicochemical data identified the compounds most likely to influence taste and preference over the applicable shelf life storage period. This analysis also yielded correlations between sensory attributes, volatile compounds and physicochemical data to determine which of these compounds and/or physicochemical data are either positively or negatively correlated with sensory attributes. Understanding the basis for these correlations could aid in the ultimate aim of this research, which is to characterise the variations in quality traits among the mapping progeny (RGxH), and thus could also assist the development of desirable traits in plant breeding programs for future strawberry production.

QTL identification for major volatile compounds

Furthermore, it would be useful to quantify the major volatile compounds linked with either desirable and undesirable sensory attributes, in particular the (E)-2-hexen-1-ol acetate (or ester, which contributes to fruity and floral notes), furaneol (or furanone, which contributes to the sweet note), and eucalyptol (or terpene, which contributes to the pine note and exhibited a high negative correlation with TSS). This work could further help in assessing preferences for samples with differing levels of taste compounds in order to correlate preference with taste perception and concentration of major volatile compounds. Ultimately, the novel

high-density SNP-based linkage map allows the mapping population to be used to identify QTL, and potentially their underlying candidate genes, which relates to the presence of major volatile compounds. Furthermore, this approach could provide new results that could then be used to help unravel aspects of metabolic pathways that have the greatest influence on taste and flavour profiles. These results could also be used to drive marker assisted selection approaches and help in developing novel strawberry varieties that are more popular and thus encourage consumers to consume a greater proportion of strawberry fruits as part of their diet.

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Appendix

Appendix 2.1. Randomisation plan of the experiment of 1st year (2013-2014).

Block	Code	Position	Block	Code	Position	Block	Code	Position
1	**Hapil**	132	1	**RG011**	608	1	**RG023**	371
2	_	626	2	_	509	2	_	292
3	_	532	3	_	439	3	_	652
4	_	346	4	_	354	4	_	706
1	**RG**	22	1	**RG012**	123	1	**RG024**	245
2	_	524	2	_	403	2	_	506
3	_	77	3	_	426	3	_	203
4	_	233	4	_	113	4	_	460
1	**RG001**	21	1	**RG013**	479	1	**RG025**	256
2	_	395	2	_	639	2	_	44
3	_	65	3	_	324	3	_	71
4	_	344	4	_	334	4	_	212
1	**RG002**	258	1	**RG014**	599	1	**RG026**	358
2	_	633	2	_	162	2	_	35
3	_	560	3	_	546	3	_	435
4	_	576	4	_	572	4	_	220
1	**RG003**	140	1	**RG015**	250	1	**RG027**	119
2	_	523	2	_	51	2	_	624
3	_	653	3	_	664	3	_	187
4	_	336	4	_	345	4	_	456
1	**RG004**	363	1	**RG016**	246	1	**RG028**	480
2	_	387	2	_	56	2	_	39
3	_	186	3	_	537	3	_	543
4	_	327	4	_	107	4	_	114
1	**RG005**	496	1	**RG017**	366	1	**RG029**	598
2	_	413	2	_	522	2	_	505
3	_	308	3	_	185	3	_	304
4	_	340	4	_	94	4	_	234
1	**RG006**	237	1	**RG018**	255	1	**RG030**	607
2	_	53	2	_	275	2	_	45
3	_	535	3	_	440	3	_	303
4	_	704	4	_	228	4	_	338
1	**RG007**	606	1	**RG019**	600	1	**RG031**	124
2	_	390	2	_	159	2	_	55
3	_	313	3	_	81	3	_	196
4	_	98	4	_	708	4	_	210
1	**RG008**	605	1	**RG020**	130	1	**RG032**	603
2	_	628	2	_	649	2	_	293

Block	Code	Position	Block	Code	Position	Block	Code	Position
3	_	423	3	_	670	3	_	314
4	_	445	4	_	585	4	_	351
1	**RG009**	592	1	**RG021**	383	1	**RG033**	2
2	_	625	2	_	512	2	_	49
3	_	663	3	_	559	3	_	658
4	_	103	4	_	116	4	_	459
1	**RG010**	13	1	**RG022**	127	1	**RG034**	241
2	_	642	2	_	384	2	_	270
3	_	320	3	_	202	3	_	416
4	_	465	4	_	343	4	_	679
1	**RG035**	379	1	**RG049**	610	1	**RG062**	9
2	_	291	2	_	32	2	_	393
3	_	322	3	_	433	3	_	200
4	_	347	4	_	566	4	_	688
1	**RG036**	239	1	**RG050**	381	1	**RG063**	380
2	_	518	2	_	155	2	_	648
3	_	417	3	_	674	3	_	419
4	_	235	4	_	224	4	_	693
1	**RG037**	492	1	**RG051**	6	1	**RG064**	252
2	_	154	2	_	647	2	_	150
3	_	188	3	_	300	3	_	298
4	_	209	4	_	451	4	_	584
1	**RG038**	259	1	**RG052**	594	1	**RG065**	365
2	_	412	2	_	268	2	_	399
3	_	178	3	_	66	3	_	533
4	_	328	4	_	226	4	_	326
1	**RG039**	613	1	**RG053**	266	1	**RG066**	618
2	_	34	2	_	394	2	_	392
3	_	542	3	_	311	3	_	553
4	_	97	4	_	686	4	_	452
1	**RG041**	378	1	**RG055**	359	1	**RG067**	138
2	_	287	2	_	529	2	_	286
3	_	306	3	_	316	3	_	424
4	_	683	4	_	698	4	_	236
1	**RG042**	18	1	**RG056**	17	1	**RG068**	370
2	_	635	2	_	504	2	_	36
3	_	195	3	_	192	3	_	421
4	_	101	4	_	95	4	_	231
1	**RG043**	120	1	**RG057**	136	1	**RG069**	597
2	_	530	2	_	280	2	_	174
3	_	422	3	_	72	3	_	309

Block	Code	Position	Block	Code	Position	Block	Code	Position
4	_	579	4	_	96	4	_	112
1	**RG045**	477	1	**RG058**	10	1	**RG070**	16
2	_	285	2	_	634	2	_	151
3	_	431	3	_	63	3	_	310
4	_	705	4	_	91	4	_	215
1	**RG046**	485	1	**RG059**	601	1	**RG071**	141
2	_	631	2	_	167	2	_	170
3	_	538	3	_	74	3	_	317
4	_	464	4	_	580	4	_	349
1	**RG047**	361	1	**RG060**	244	1	**RG072**	3
2	_	407	2	_	503	2	_	526
3	_	194	3	_	418	3	_	657
4	_	353	4	_	700	4	_	90
1	**RG048**	498	1	**RG061**	125	1	**RG073**	261
2	_	168	2	_	517	2	_	398
3	_	651	3	_	668	3	_	181
4	_	692	4	_	99	4	_	447
1	**RG074**	473	1	**RG086**	490	1	**RG100**	139
2	_	33	2	_	644	2	_	153
3	_	556	3	_	179	3	_	198
4	_	470	4	_	337	4	_	213
1	**RG075**	593	1	**RG087**	11	1	**RG102**	23
2	_	397	2	_	643	2	_	58
3	_	672	3	_	68	3	_	296
4	_	332	4	_	583	4	_	109
1	**RG076**	373	1	**RG088**	243	1	**RG103**	500
2	_	169	2	_	271	2	_	294
3	_	547	3	_	650	3	_	83
4	_	227	4	_	680	4	_	577
1	**RG077**	619	1	**RG089**	8	1	**RG104**	128
2	_	161	2	_	521	2	_	630
3	_	206	3	_	673	3	_	184
4	_	225	4	_	685	4	_	461
1	**RG078**	377	1	**RG091**	487	1	**RG106**	362
2	_	636	2	_	163	2	_	282
3	_	183	3	_	656	3	_	84
4	_	581	4	_	92	4	_	689
1	**RG079**	7	1	**RG092**	481	1	**RG107**	364
2	_	404	2	_	620	2	_	637
3	_	67	3	_	318	3	_	307
4	_	463	4	_	697	4	_	578

Block	Code	Position	Block	Code	Position	Block	Code	Position
1	**RG080**	355	1	**RG093**	133	1	**RG108**	484
2	_	510	2	_	281	2	_	645
3	_	191	3	_	665	3	_	437
4	_	563	4	_	569	4	_	564
1	**RG081**	134	1	**RG094**	122	1	**RG109**	251
2	_	531	2	_	385	2	_	152
3	_	554	3	_	545	3	_	325
4	_	574	4	_	467	4	_	571
1	**RG082**	265	1	**RG096**	254	1	**RG110**	374
2	_	54	2	_	279	2	_	278
3	_	654	3	_	312	3	_	555
4	_	100	4	_	699	4	_	687
1	**RG083**	612	1	**RG097**	143	1	**RG111**	144
2	_	43	2	_	290	2	_	408
3	_	558	3	_	660	3	_	676
4	_	105	4	_	449	4	_	335
1	**RG084**	30	1	**RG098**	121	1	**RG112**	615
2	_	272	2	_	641	2	_	508
3	_	299	3	_	552	3	_	190
4	_	342	4	_	589	4	_	588
1	**RG085**	489	1	**RG099**	5	1	**RG113**	493
2	_	172	2	_	514	2	_	50
3	_	315	3	_	61	3	_	430
4	_	570	4	_	568	4	_	454
1	**RG115**	19	1	**RG127**	488	1	**RG140**	482
2	_	156	2	_	46	2	_	165
3	_	182	3	_	321	3	_	659
4	_	690	4	_	102	4	_	701
1	**RG116**	499	1	**RG128**	602	1	**RG141**	360
2	_	410	2	_	276	2	_	176
3	_	201	3	_	302	3	_	539
4	_	455	4	_	111	4	_	339
1	**RG117**	12	1	**RG129**	146	1	**RG142**	137
2	_	402	2	_	640	2	_	269
3	_	415	3	_	536	3	_	420
4	_	684	4	_	453	4	_	703
1	**RG118**	369	1	**RG130**	478	1	**RG143**	502
2	_	42	2	_	149	2	_	391
3	_	662	3	_	85	3	_	427
4	_	218	4	_	217	4	_	331
1	**RG119**	242	1	**RG132**	475	1	**RG144**	29

Block	Code	Position	Block	Code	Position	Block	Code	Position
2	_	148	2	_	507	2	_	37
3	_	89	3	_	677	3	_	441
4	_	350	4	_	352	4	_	222
1	**RG120**	15	1	**RG133**	595	1	**RG145**	25
2	_	289	2	_	171	2	_	388
3	_	540	3	_	678	3	_	75
4	_	230	4	_	457	4	_	471
1	**RG121**	382	1	**RG134**	147	1	**RG146**	253
2	_	411	2	_	638	2	_	629
3	_	675	3	_	193	3	_	189
4	_	696	4	_	211	4	_	458
1	**RG122**	375	1	**RG135**	614	1	**RG147**	495
2	_	627	2	_	400	2	_	513
3	_	197	3	_	534	3	_	425
4	_	219	4	_	444	4	_	223
1	**RG123**	247	1	**RG136**	486	1	**RG148**	240
2	_	48	2	_	622	2	_	277
3	_	323	3	_	561	3	_	70
4	_	694	4	_	232	4	_	695
1	**RG124**	262	1	**RG137**	28	1	**RG149**	27
2	_	401	2	_	519	2	_	52
3	_	414	3	_	432	3	_	551
4	_	208	4	_	104	4	_	448
1	**RG125**	14	1	**RG138**	596	1	**RG150**	264
2	_	623	2	_	525	2	_	632
3	_	301	3	_	669	3	_	655
4	_	586	4	_	575	4	_	118
1	**RG126**	474	1	**RG139**	367	1	**RG151**	368
2	_	177	2	_	38	2	_	527
3	_	69	3	_	544	3	_	549
4	_	707	4	_	93	4	_	229
1	**RG152**	376	1	**RG168**	609	1	**RG180**	131
2	_	160	2	_	621	2	_	516
3	_	87	3	_	429	3	_	442
4	_	330	4	_	207	4	_	468
1	**RG153**	591	1	**RG169**	126	1	**RG181**	24
2	_	267	2	_	41	2	_	284
3	_	557	3	_	671	3	_	60
4	_	333	4	_	106	4	_	216
1	**RG155**	604	1	**RG170**	4	1	**RG182**	238
2	_	396	2	_	59	2	_	646

Block	Code	Position	Block	Code	Position	Block	Code	Position
3	_	661	3	_	667	3	_	305
4	_	443	4	_	682	4	_	110
1	**RG157**	491	1	**RG171**	135	1	**RG183**	616
2	_	273	2	_	166	2	_	157
3	_	78	3	_	204	3	_	86
4	_	466	4	_	214	4	_	450
1	**RG158**	611	1	**RG172**	497	1	**RG184**	142
2	_	164	2	_	158	2	_	511
3	_	436	3	_	79	3	_	297
4	_	681	4	_	117	4	_	348
1	**RG159**	260	1	**RG173**	356	1	**RG185**	357
2	_	283	2	_	288	2	_	31
3	_	428	3	_	438	3	_	319
4	_	108	4	_	582	4	_	691
1	**RG160**	483	1	**RG174**	145	1	**RG186**	1
2	_	405	2	_	57	2	_	40
3	_	205	3	_	666	3	_	80
4	_	462	4	_	115	4	_	573
1	**RG161**	617	1	**RG175**	129	1	**RG187**	248
2	_	409	2	_	406	2	_	175
3	_	550	3	_	76	3	_	541
4	_	329	4	_	221	4	_	446
1	**RG162**	26	1	**RG176**	372	1	**RG188**	263
2	_	389	2	_	47	2	_	528
3	_	73	3	_	548	3	_	64
4	_	469	4	_	565	4	_	472
1	**RG163**	20	1	**RG177**	249			
2	_	520	2	_	386			
3	_	82	3	_	199			
4	_	702	4	_	590			
1	**RG164**	494	1	**RG178**	476			
2	_	173	2	_	274			
3	_	62	3	_	434			
4	_	341	4	_	587			
1	**RG167**	257	1	**RG179**	501			
2	_	295	2	_	515			
3	_	88	3	_	180			
4	_	567	4	_	562			

Appendix 2.2. Randomisation plan of the experiment of 2nd year (2014-2015).

Block	Code	Position	Block	Code	Position
1	**Hapil**	23	1	**RG092**	45
1	_	92	1	_	8
2	_	43	2	_	23
2	_	114	2	_	104
1	**Redgntlt**	5	1	**RG093**	113
1	_	93	1	_	74
2	_	134	2	_	65
2	_	92	2	_	101
1	**RG001**	117	1	**RG096**	90
1	_	104	1	_	5
2	_	59	2	_	66
2	_	105	2	_	93
1	**RG002**	7	1	**RG097**	75
1	_	43	1	_	127
2	_	7	2	_	124
2	_	67	2	_	115
1	**RG003**	97	1	**RG098**	119
1	_	9	1	_	99
2	_	138	2	_	64
2	_	76	2	_	25
1	**RG004**	70	1	**RG099**	122
1	_	26	1	_	49
2	_	25	2	_	120
2	_	42	2	_	121
1	**RG005**	6	1	**RG100**	99
1	_	113	1	_	100
2	_	85	2	_	76
2	_	94	2	_	100
1	**RG006**	62	1	**RG102**	103
1	_	30	1	_	129
2	_	94	2	_	50
2	_	55	2	_	64
1	**RG007**	32	1	**RG106**	57
1	_	29	1	_	67
2	_	135	2	_	4
2	_	123	2	_	66
1	**RG008**	139	1	**RG107**	91
1	_	65	1	_	23
2	_	132	2	_	54

Block	Code	Position	Block	Code	Position
2	_	140	2	_	75
1	**RG010**	43	1	**RG108**	82
1	_	27	1	_	19
2	_	44	2	_	90
2	_	14	2	_	4
1	**RG011**	39	1	**RG109**	86
1	_	87	1	_	137
2	_	30	2	_	111
2	_	49	2	_	145
1	**RG012**	145	1	**RG110**	128
1	_	63	1	_	83
2	_	107	2	_	139
2	_	144	2	_	24
1	**RG013**	104	1	**RG111**	74
1	_	73	1	_	147
2	_	112	2	_	21
2	_	57	2	_	130
1	**RG014**	63	1	**RG112**	17
1	_	69	1	_	123
2	_	42	2	_	126
2	_	18	2	_	139
1	**RG015**	124	1	**RG115**	38
1	_	118	1	_	103
2	_	26	2	_	68
2	_	103	2	_	7
1	**RG017**	50	1	**RG116**	114
1	_	64	1	_	75
2	_	2	2	_	39
2	_	102	2	_	38
1	**RG018**	110	1	**RG117**	27
1	_	136	1	_	110
2	_	9	2	_	69
2	_	48	2	_	73
1	**RG020**	10	1	**RG118**	141
1	_	143	1	_	78
2	_	141	2	_	84
2	_	59	2	_	125
1	**RG021**	133	1	**RG119**	135
1	_	33	1	_	101
2	_	75	2	_	52
2	_	62	2	_	129

Block	Code	Position	Block	Code	Position
1	**RG023**	134	1	**RG120**	51
1	_	46	1	_	140
2	_	81	2	_	78
2	_	97	2	_	51
1	**RG024**	95	1	**RG121**	48
1	_	35	1	_	124
2	_	51	2	_	87
2	_	79	2	_	5
1	**RG026**	49	1	**RG122**	54
1	_	132	1	_	139
2	_	24	2	_	83
2	_	71	2	_	65
1	**RG027**	67	1	**RG124**	144
1	_	51	1	_	107
2	_	11	2	_	58
2	_	15	2	_	39
1	**RG028**	85	1	**RG125**	2
1	_	28	1	_	144
2	_	125	2	_	56
2	_	11	2	_	16
1	**RG029**	52	1	**RG126**	146
1	_	81	1	_	52
2	_	12	2	_	109
2	_	87	2	_	120
1	**RG030**	46	1	**RG127**	53
1	_	119	1	_	108
2	_	32	2	_	67
2	_	41	2	_	126
1	**RG031**	125	1	**RG129**	33
1	_	31	1	_	91
2	_	57	2	_	61
2	_	29	2	_	19
1	**RG033**	118	1	**RG130**	3
1	_	80	1	_	32
2	_	46	2	_	45
2	_	116	2	_	63
1	**RG035**	64	1	**RG132**	71
1	_	82	1	_	39
2	_	20	2	_	82
2	_	40	2	_	95
1	**RG037**	22	1	**RG134**	12

Block	Code	Position	Block	Code	Position
1	_	142	1	_	3
2	_	38	2	_	117
2	_	12	2	_	113
1	**RG038**	18	1	**RG136**	26
1	_	36	1	_	141
2	_	80	2	_	72
2	_	54	2	_	28
1	**RG039**	88	1	**RG137**	55
1	_	62	1	_	44
2	_	3	2	_	104
2	_	1	2	_	131
1	**RG041**	69	1	**RG138**	1
1	_	98	1	_	53
2	_	140	2	_	142
2	_	52	2	_	138
1	**RG042**	83	1	**RG139**	116
1	_	58	1	_	96
2	_	77	2	_	99
2	_	111	2	_	98
1	**RG043**	77	1	**RG140**	93
1	_	126	1	_	13
2	_	29	2	_	8
2	_	50	2	_	70
1	**RG045**	129	1	**RG141**	89
1	_	14	1	_	95
2	_	59	2	_	27
2	_	43	2	_	136
1	**RG046**	40	1	**RG142**	130
1	_	42	1	_	146
2	_	113	2	_	118
2	_	22	2	_	31
1	**RG047**	47	1	**RG143**	31
1	_	1	1	_	117
2	_	36	2	_	143
2	_	142	2	_	72
1	**RG048**	36	1	**RG144**	14
1	_	115	1	_	68
2	_	119	2	_	73
2	_	21	2	_	110
1	**RG049**	112	1	**RG145**	111
1	_	90	1	_	55

Block	Code	Position	Block	Code	Position
2	_	101	2	_	121
2	_	3	2	_	47
1	**RG051**	34	1	**RG146**	28
1	_	60	1	_	130
2	_	36	2	_	136
2	_	58	2	_	90
1	**RG053**	16	1	**RG147**	121
1	_	71	1	_	12
2	_	34	2	_	48
2	_	91	2	_	17
1	**RG054**	35	1	**RG148**	142
1	_	6	1	_	22
2	_	37	2	_	96
2	_	137	2	_	9
1	**RG055**	84	1	**RG149**	72
1	_	10	1	_	120
2	_	97	2	_	102
2	_	109	2	_	88
1	**RG056**	101	1	**RG150**	61
1	_	116	1	_	70
2	_	28	2	_	10
2	_	23	2	_	8
1	**RG057**	136	1	**RG153**	92
1	_	20	1	_	66
2	_	146	2	_	5
2	_	34	2	_	141
1	**RG058**	127	1	**RG156**	106
1	_	41	1	_	94
2	_	62	2	_	7
2	_	112	2	_	53
1	**RG060**	108	1	**RG158**	56
1	_	112	1	_	47
2	_	14	2	_	144
2	_	26	2	_	134
1	**RG061**	112	1	**RG159**	60
1	_	104	1	_	76
2	_	147	2	_	30
2	_	69	2	_	2
1	**RG062**	58	1	**RG161**	73
1	_	109	1	_	121
2	_	110	2	_	16

Block	Code	Position	Block	Code	Position
2	_	85	2	_	127
1	**RG063**	120	1	**RG162**	13
1	_	138	1	_	56
2	_	100	2	_	91
2	_	6	2	_	61
1	**RG064**	21	1	**RG163**	15
1	_	145	1	_	21
2	_	89	2	_	79
2	_	46	2	_	107
1	**RG065**	44	1	**RG164**	102
1	_	45	1	_	77
2	_	35	2	_	17
2	_	60	2	_	32
1	**RG066**	80	1	**RG167**	123
1	_	68	1	_	15
2	_	55	2	_	128
2	_	27	2	_	117
1	**RG067**	131	1	**RG168**	117
1	_	48	1	_	79
2	_	13	2	_	129
2	_	84	2	_	13
1	**RG068**	68	1	**RG169**	79
1	_	128	1	_	61
2	_	33	2	_	88
2	_	10	2	_	143
1	**RG069**	37	1	**RG170**	140
1	_	59	1	_	16
2	_	53	2	_	19
2	_	77	2	_	74
1	**RG071**	24	1	**RG171**	42
1	_	97	1	_	50
2	_	70	2	_	71
2	_	119	2	_	124
1	**RG072**	107	1	**RG172**	115
1	_	106	1	_	57
2	_	130	2	_	6
2	_	36	2	_	107
1	**RG073**	78	1	**RG173**	138
1	_	133	1	_	86
2	_	15	2	_	1
2	_	81	2	_	135

Block	Code	Position	Block	Code	Position
1	**RG074**	126	1	**RG174**	87
1	_	72	1	_	105
2	_	47	2	_	49
2	_	128	2	_	83
1	**RG075**	81	1	**RG175**	41
1	_	85	1	_	84
2	_	18	2	_	22
2	_	133	2	_	122
1	**RG077**	11	1	**RG177**	8
1	_	131	1	_	2
2	_	116	2	_	131
2	_	86	2	_	99
1	**RG078**	25	1	**RG178**	147
1	_	111	1	_	89
2	_	40	2	_	122
2	_	37	2	_	80
1	**RG079**	59	1	**RG179**	19
1	_	102	1	_	54
2	_	114	2	_	93
2	_	118	2	_	56
1	**RG080**	143	1	**RG180**	109
1	_	37	1	_	24
2	_	103	2	_	105
2	_	33	2	_	44
1	**RG083**	9	1	**RG181**	132
1	_	114	1	_	135
2	_	108	2	_	86
2	_	78	2	_	82
1	**RG084**	20	1	**RG182**	137
1	_	4	1	_	18
2	_	115	2	_	123
2	_	89	2	_	96
1	**RG085**	96	1	**RG184**	105
1	_	34	1	_	134
2	_	92	2	_	31
2	_	106	2	_	45
1	**RG086**	94	1	**RG185**	98
1	_	125	1	_	38
2	_	60	2	_	133
2	_	147	2	_	35
1	**RG088**	76	1	**RG186**	65

Block	Code	Position	Block	Code	Position
1	_	11	1	_	122
2	_	95	2	_	41
2	_	108	2	_	103
1	**RG089**	4	1	**RG187**	30
1	_	40	1	_	88
2	_	63	2	_	74
2	_	132	2	_	20
1	**RG091**	66	1		
1	_	25	1		
2	_	137	2		
2	_	68	2		

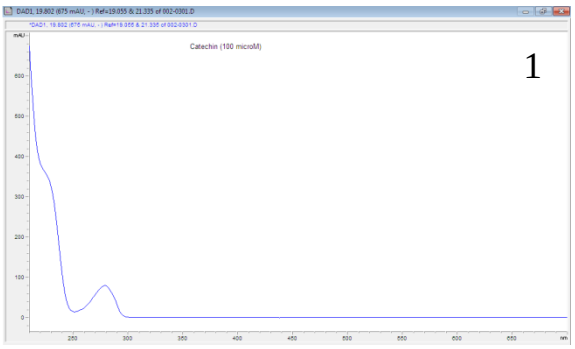
Appendix 2.3. Randomisation plan of the experiment of 3rd year (2015-2016).

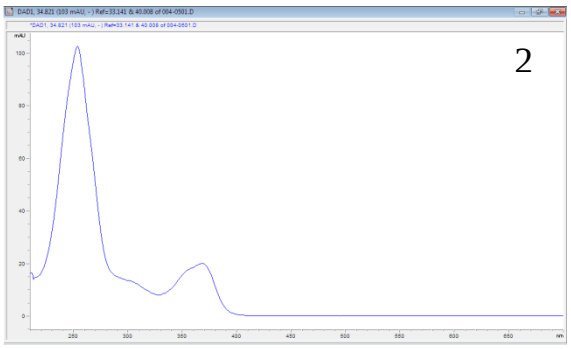
In block	Block 1	Block 2	Block 3
1	RG164	RG086	RG098
2	Hapil	RG126	RG121
3	RG010	RG100	RG121
4	RG098	RG098	RG098
5	RG100	RG	RG086
6	Hapil	RG010	Hapil
7	RG098	RG169	RG
8	Hapil	RG098	RG121
9	RG169	RG010	RG010
10	RG098	RG010	RG169
11	RG010	RG100	Hapil
12	Hapil	RG121	RG126
13	RG	RG164	Hapil
14	RG121	Hapil	RG169
15	RG098	Hapil	RG169
16	RG164	RG010	RG100
17	RG126	RG	RG121
18	RG	RG121	RG086
19	RG010	RG100	RG086
20	RG121	RG086	RG086
21	RG164	Hapil	RG010
22	RG098	RG169	RG
23	RG010	RG	RG121
24	RG164	RG164	RG100
25	RG126	RG164	RG164
26	RG121	RG121	RG
27	RG086	RG126	RG164
28	RG098	Hapil	RG126
29	RG121	RG126	RG164
30	RG121	Hapil	RG
31	RG164	Hapil	RG098
32	RG100	RG100	RG126
33	RG169	RG126	RG100
34	RG	RG086	RG126
35	RG100	RG169	RG121
36	RG086	RG	RG086
37	RG169	RG121	RG010
38	RG100	RG100	RG
39	RG169	RG126	RG126
40	RG086	RG098	RG100
41	RG100	RG	RG100
42	RG126	RG010	RG100

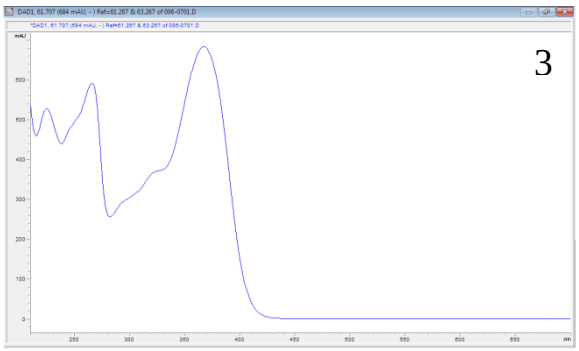
In block	Block 1	Block 2	Block 3
43	RG126	Hapil	RG098
44	RG	RG100	RG
45	RG126	RG098	RG169
46	RG010	RG098	RG169
47	RG169	RG098	RG098
48	RG126	RG164	RG098
49	RG126	RG100	RG010
50	RG010	RG169	RG121
51	Hapil	RG	RG010
52	RG086	RG169	RG086
53	RG086	RG	RG098
54	RG126	RG098	RG164
55	RG	RG086	RG010
56	RG100	RG	RG086
57	RG010	RG010	RG126
58	RG086	RG121	RG121
59	RG	RG121	RG164
60	RG	RG121	RG164
61	RG100	RG086	RG086
62	RG169	RG086	RG126
63	RG121	RG100	RG169
64	RG	RG126	RG164
65	RG086	Hapil	Hapil
66	RG121	RG164	RG100
67	RG086	RG164	RG086
68	RG169	RG164	RG164
69	Hapil	RG169	Hapil
70	RG164	RG169	RG169
71	RG121	RG	RG126
72	Hapil	RG164	Hapil
73	RG121	RG126	Hapil
74	RG010	RG010	RG126
75	RG121	RG086	RG098
76	RG098	RG010	RG010
77	Hapil	RG086	Hapil
78	RG010	RG010	Hapil
79	RG086	RG164	RG098
80	Hapil	RG126	Hapil
81	RG098	RG086	RG169
82	RG169	RG169	RG100
83	RG164	RG098	RG121
84	RG169	RG	RG098
85	RG169	RG169	RG

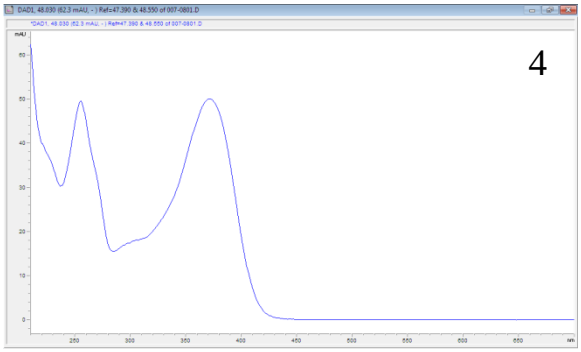
In block	Block 1	Block 2	Block 3
86	RG086	RG164	RG
87	RG	RG098	RG100
88	RG164	RG121	RG010
89	RG126	RG010	RG169
90	RG100	RG126	RG086
91	RG100	RG086	RG010
92	RG098	RG169	RG
93	RG010	Hapil	RG164
94	RG126	RG100	RG164
95	RG164	RG121	RG010
96	RG164	RG126	RG
97	Hapil	RG098	RG100
98	RG100	Hapil	RG126
99	RG	RG121	RG121
100	RG098	RG100	RG169

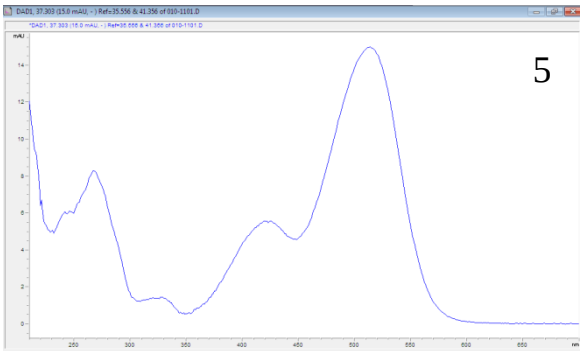
Appendix 3.1. The spectrum of the polyphenols' standards dissolved in HPLC-grade methanol; 1) Catechin 100 µm, 2) Ellagic acid 100 µm, 3) Kaempferol 100 µm, 4) Quercetin 50 µm, 5) Pelargonidin 100 µm, and 7) Cyanidin 100 µm. (Folder name: RA141013).

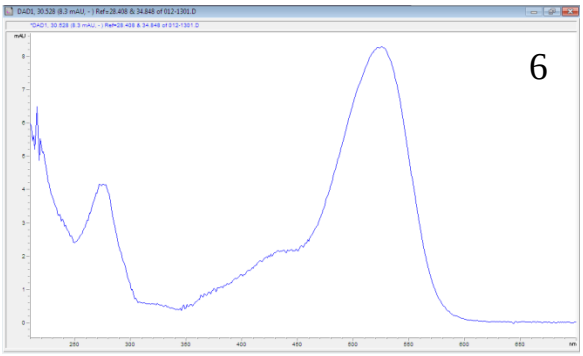




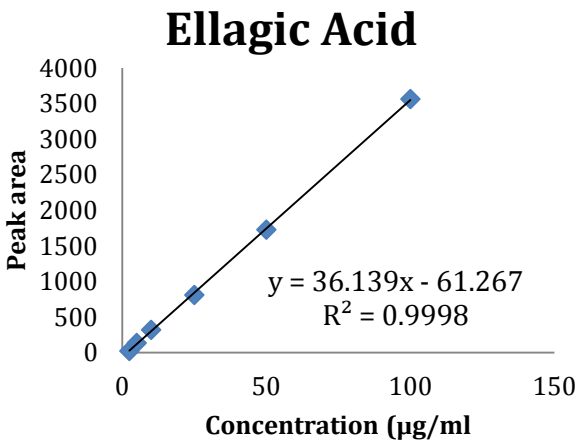


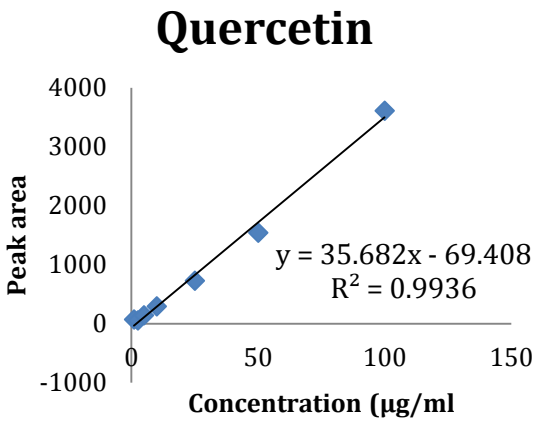


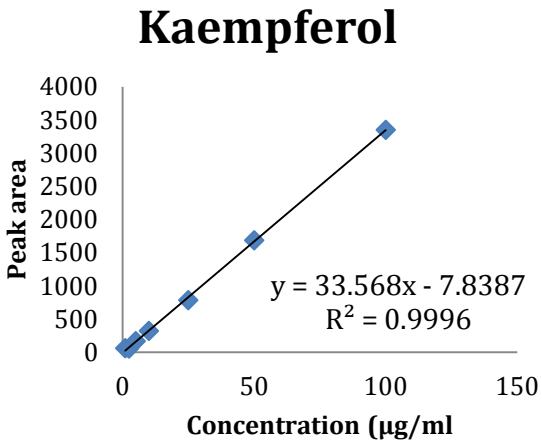


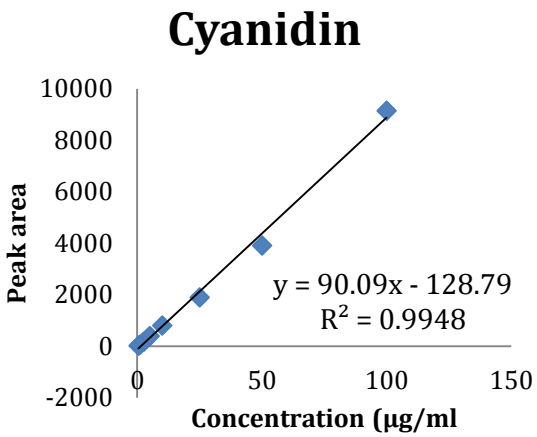


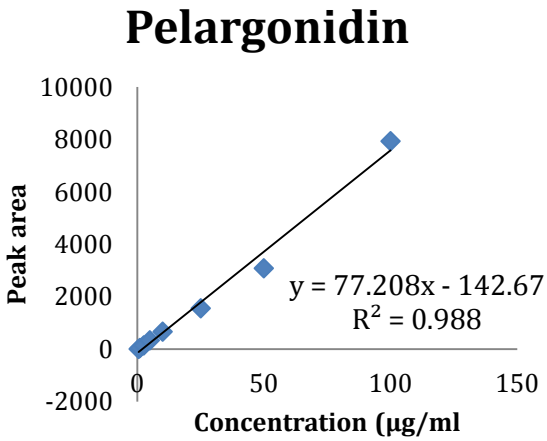
Appendix 3.2. Calibration curves of standards diluted in mobile phase A.



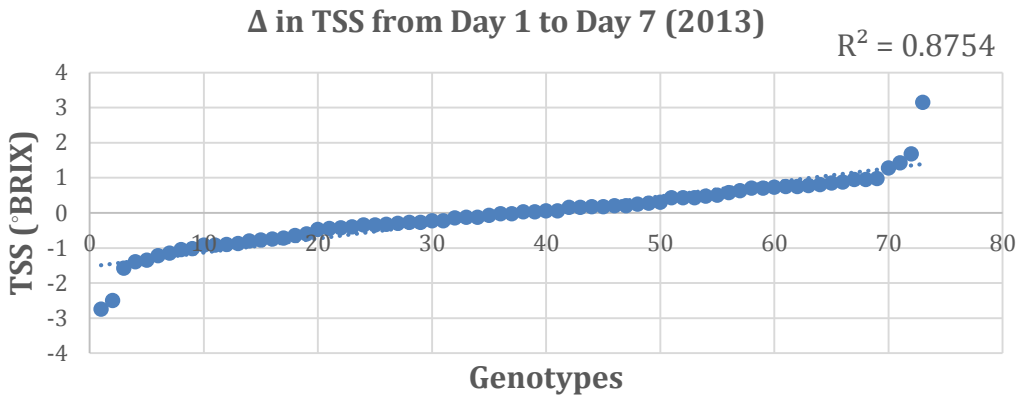


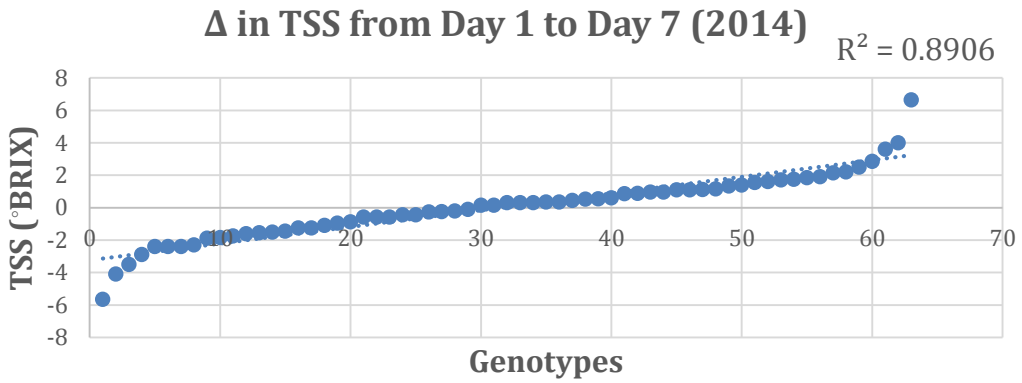


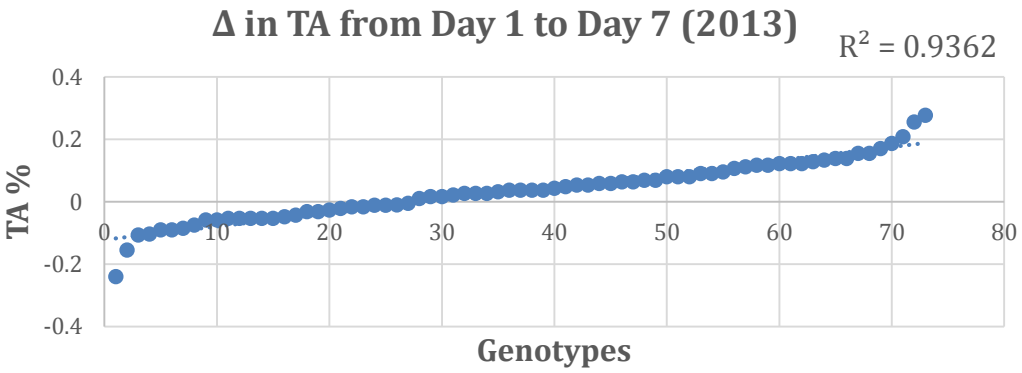


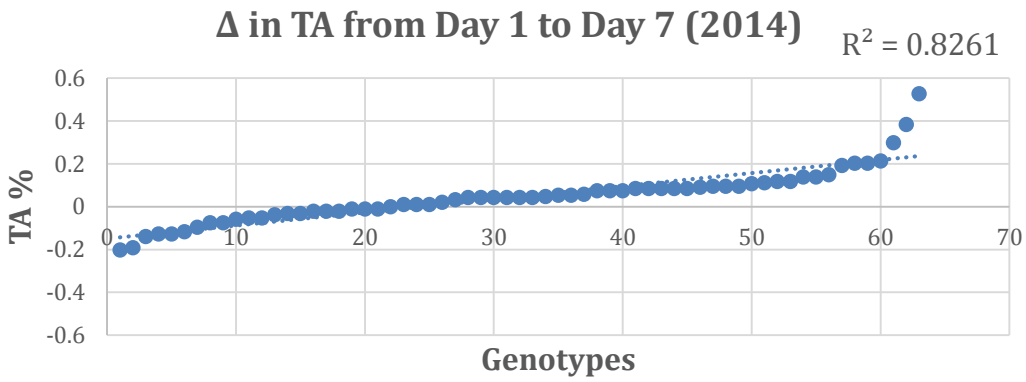


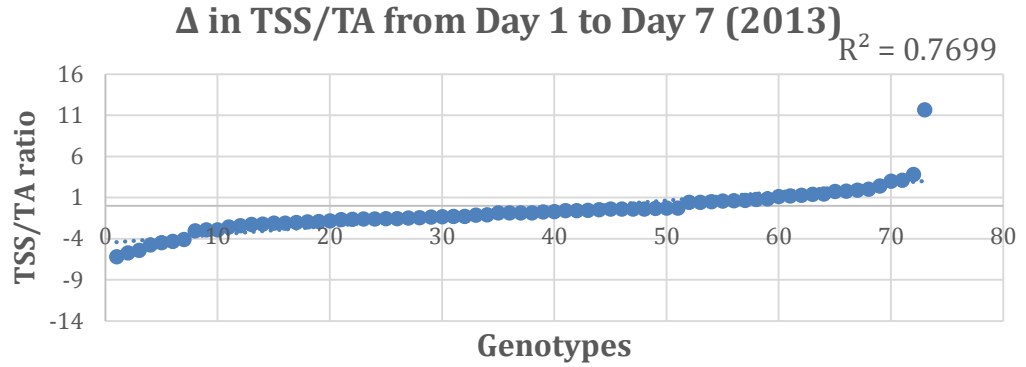
Appendix 3.3. Scatter plots for changes from day 1 to day 7 in all quality traits.

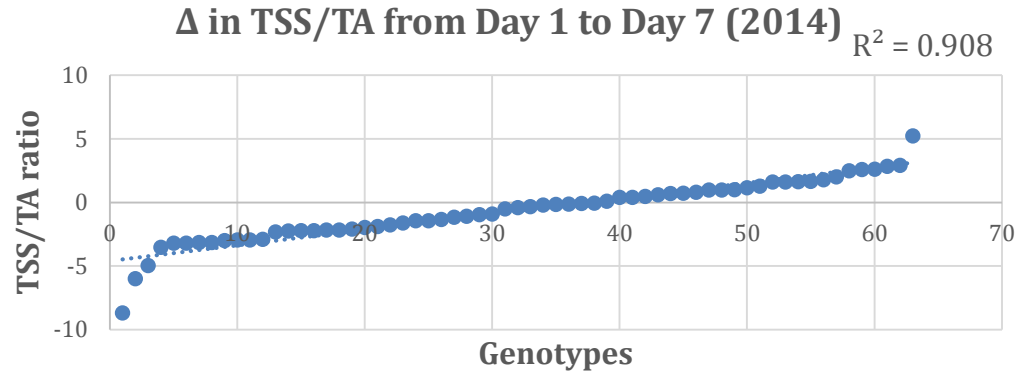


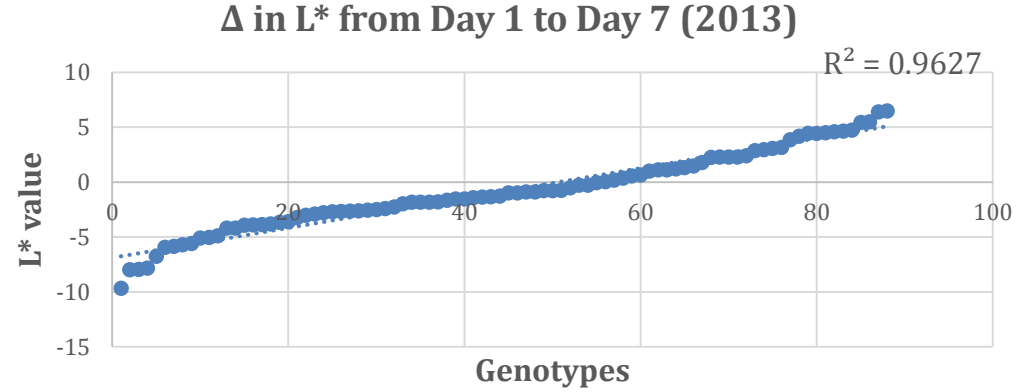


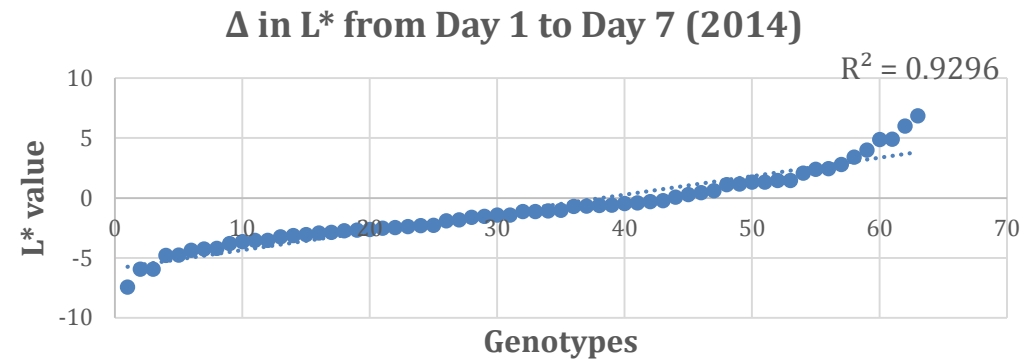


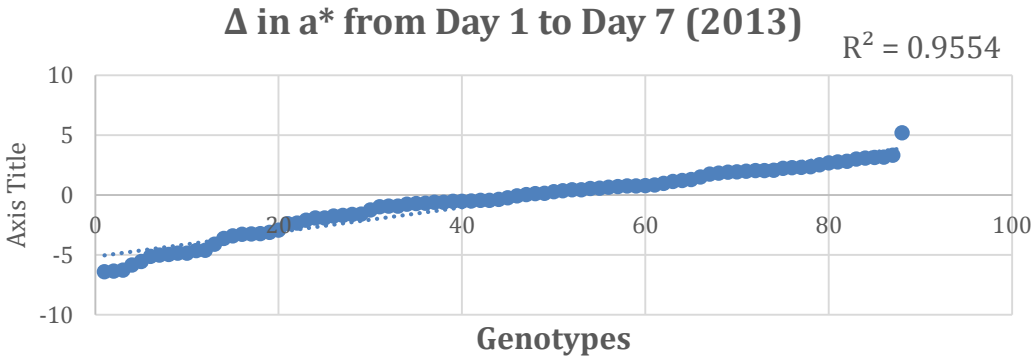

Δ in TA from Day 1 to Day 7 (2014)
R² = 0.8261
TA %
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
0
10
20
30
40
50
60
70
Genotypes

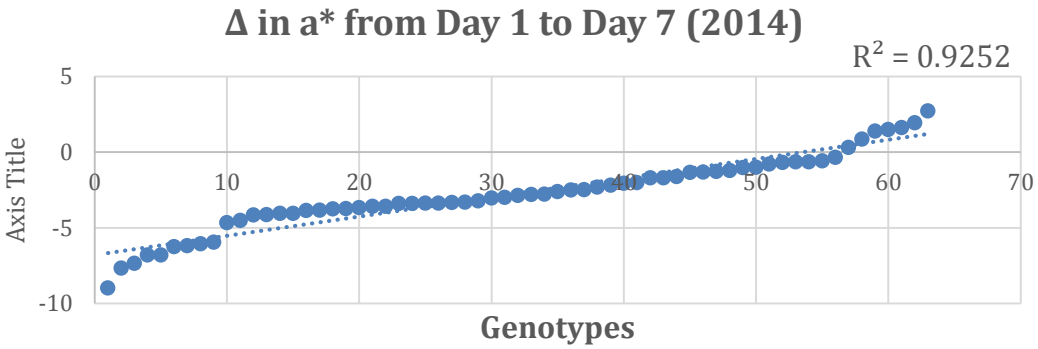

Δ in TSS/TA from Day 1 to Day 7 (2013)
R² = 0.7699
TSS/TA ratio
16
11
6
1
-4
-9
-14
0
10
20
30
40
50
60
70
80
Genotypes

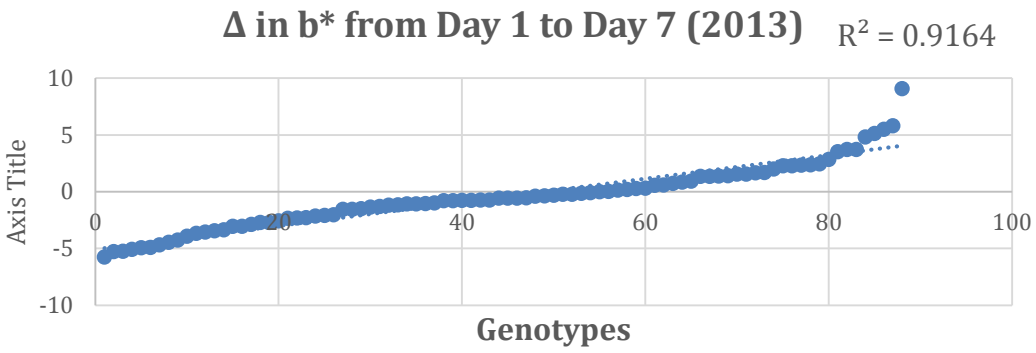

Δ in TSS/TA from Day 1 to Day 7 (2014)
R² = 0.908
TSS/TA ratio
10
5
0
-5
-10
0
10
20
30
40
50
60
70
Genotypes

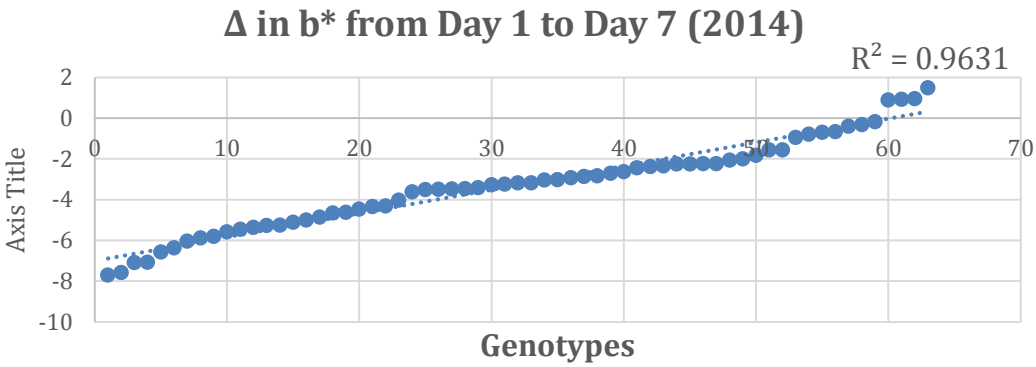

Δ in L* from Day 1 to Day 7 (2013)
R² = 0.9627
L* value
10
5
0
-5
-10
-15
0
20
40
60
80
100
Genotypes

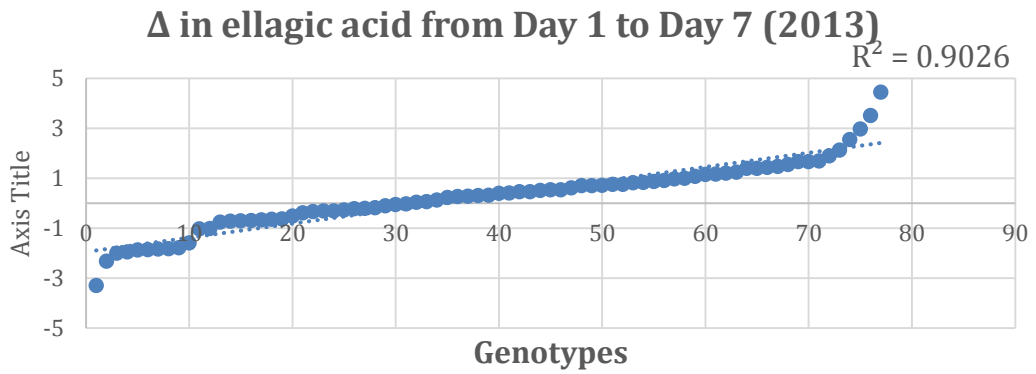

Δ in L* from Day 1 to Day 7 (2014)
R² = 0.9296
L* value
10
5
0
-5
-10
0
10
20
30
40
50
60
70
Genotypes

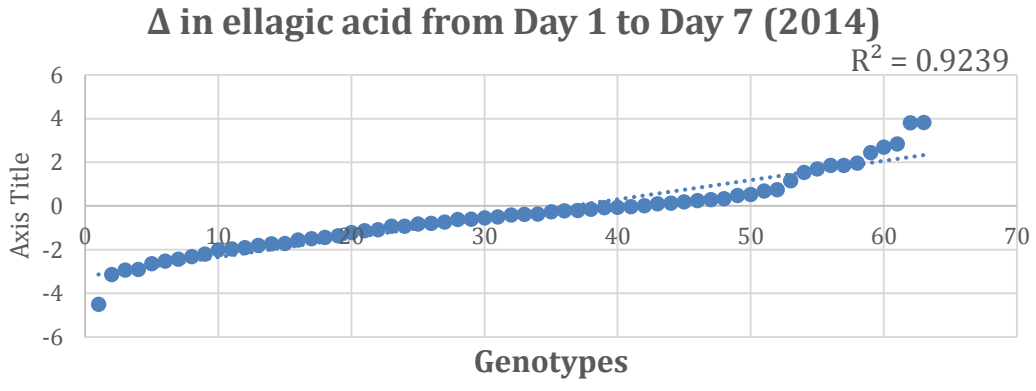

Δ in a* from Day 1 to Day 7 (2013)
R² = 0.9554
Axis Title
10
5
0
-5
-10
0
20
40
60
80
100
Genotypes

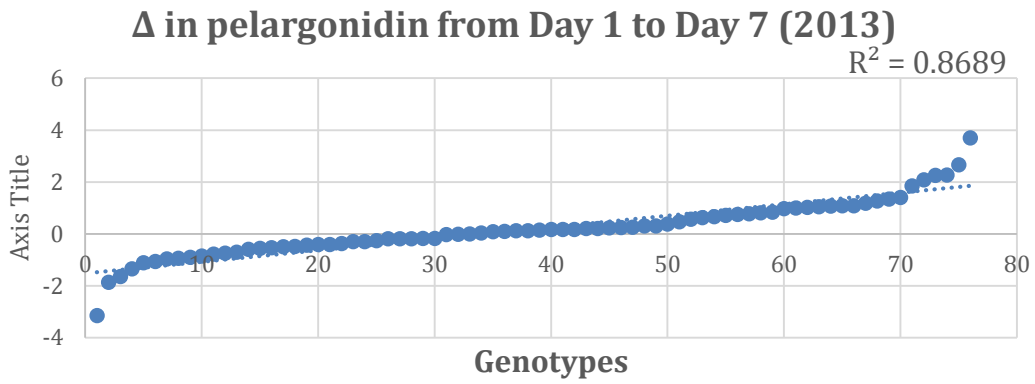

Δ in a* from Day 1 to Day 7 (2014)
R² = 0.9252
Axis Title
5
0
-5
-10
0
10
20
30
40
50
60
70
Genotypes

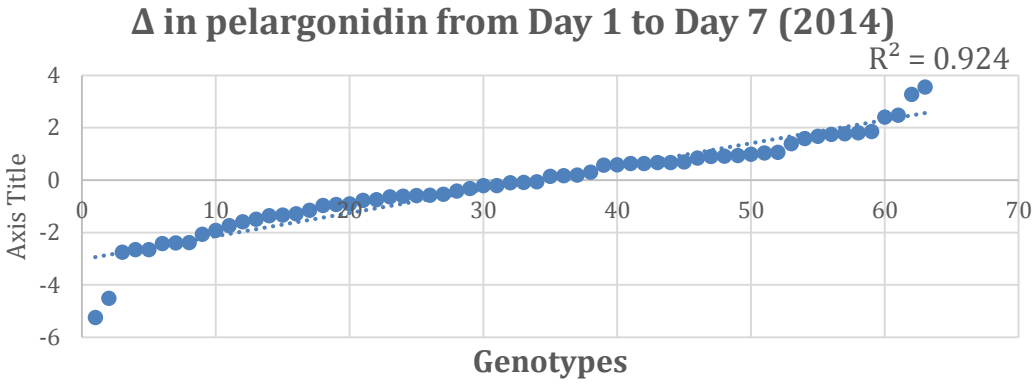

Δ in b* from Day 1 to Day 7 (2013)
R² = 0.9164
Axis Title
10
5
0
-5
-10
0
20
40
60
80
100
Genotypes

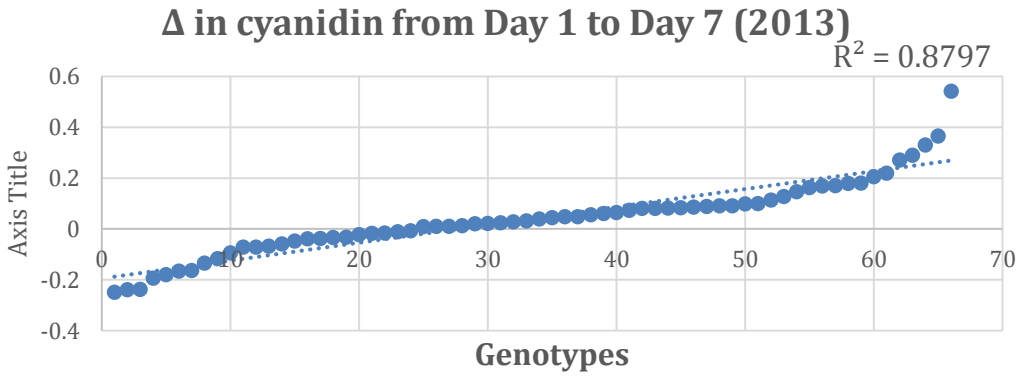

Δ in b* from Day 1 to Day 7 (2014)
R² = 0.9631
Axis Title
2
0
-2
-4
-6
-8
-10
0
10
20
30
40
50
60
70
Genotypes

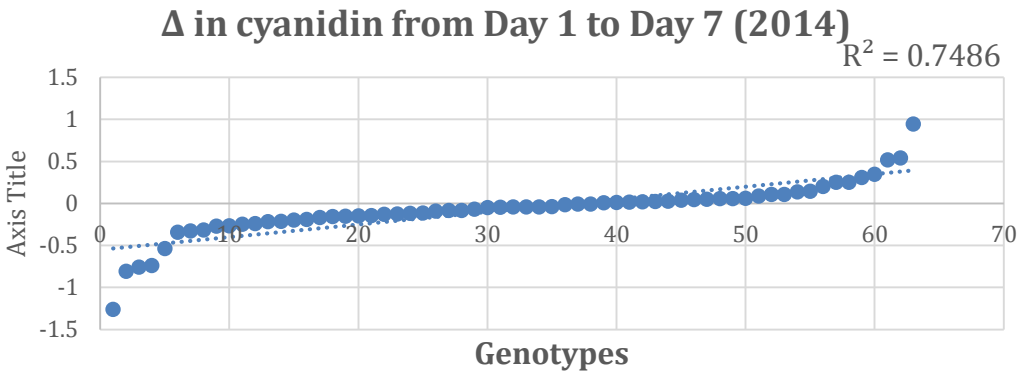

Δ in ellagic acid from Day 1 to Day 7 (2013)
R² = 0.9026
Axis Title
5
3
1
-1
-3
-5
0
10
20
30
40
50
60
70
80
90
Genotypes

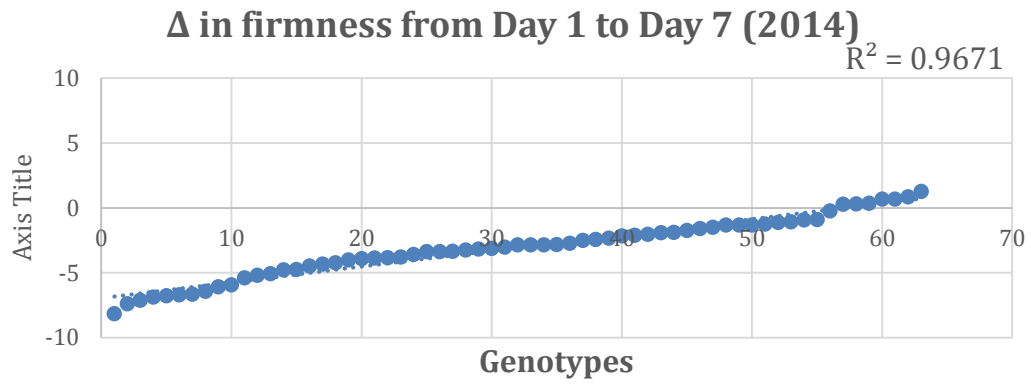

Δ in ellagic acid from Day 1 to Day 7 (2014)
R² = 0.9239
Axis Title
6
4
2
0
-2
-4
-6
0
10
20
30
40
50
60
70
Genotypes

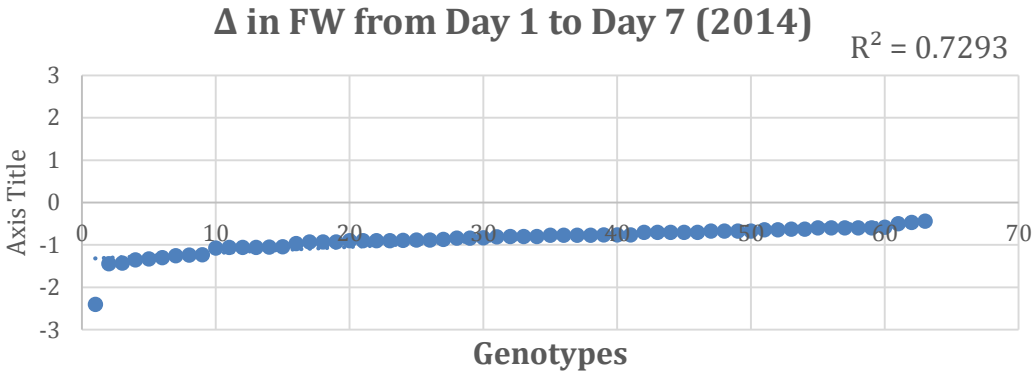

Δ in pelargonidin from Day 1 to Day 7 (2013)
R² = 0.8689
Axis Title
Genotypes


Δ in pelargonidin from Day 1 to Day 7 (2014)
R² = 0.924
Axis Title
Genotypes

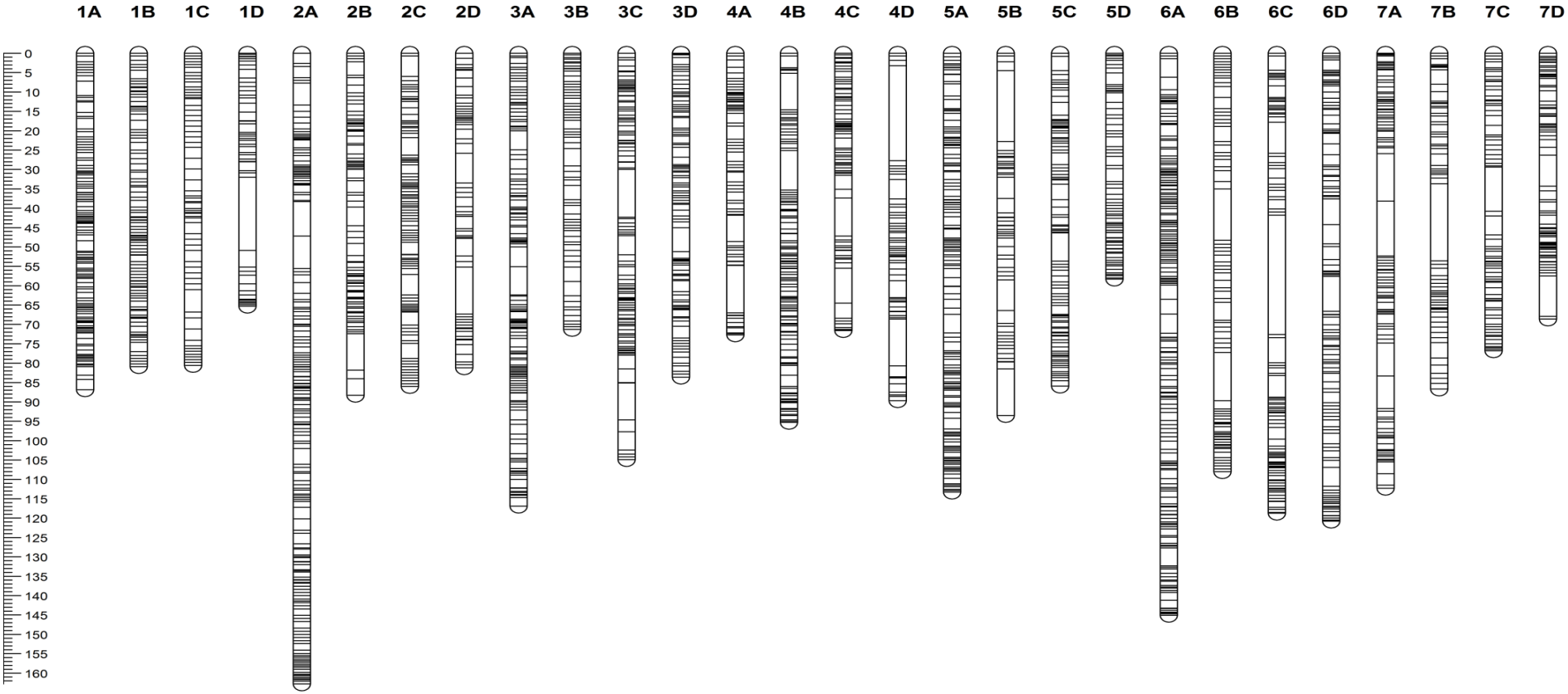

Δ in cyanidin from Day 1 to Day 7 (2013)
R² = 0.8797
Axis Title
Genotypes


Δ in cyanidin from Day 1 to Day 7 (2014)
R² = 0.7486
Axis Title
Genotypes

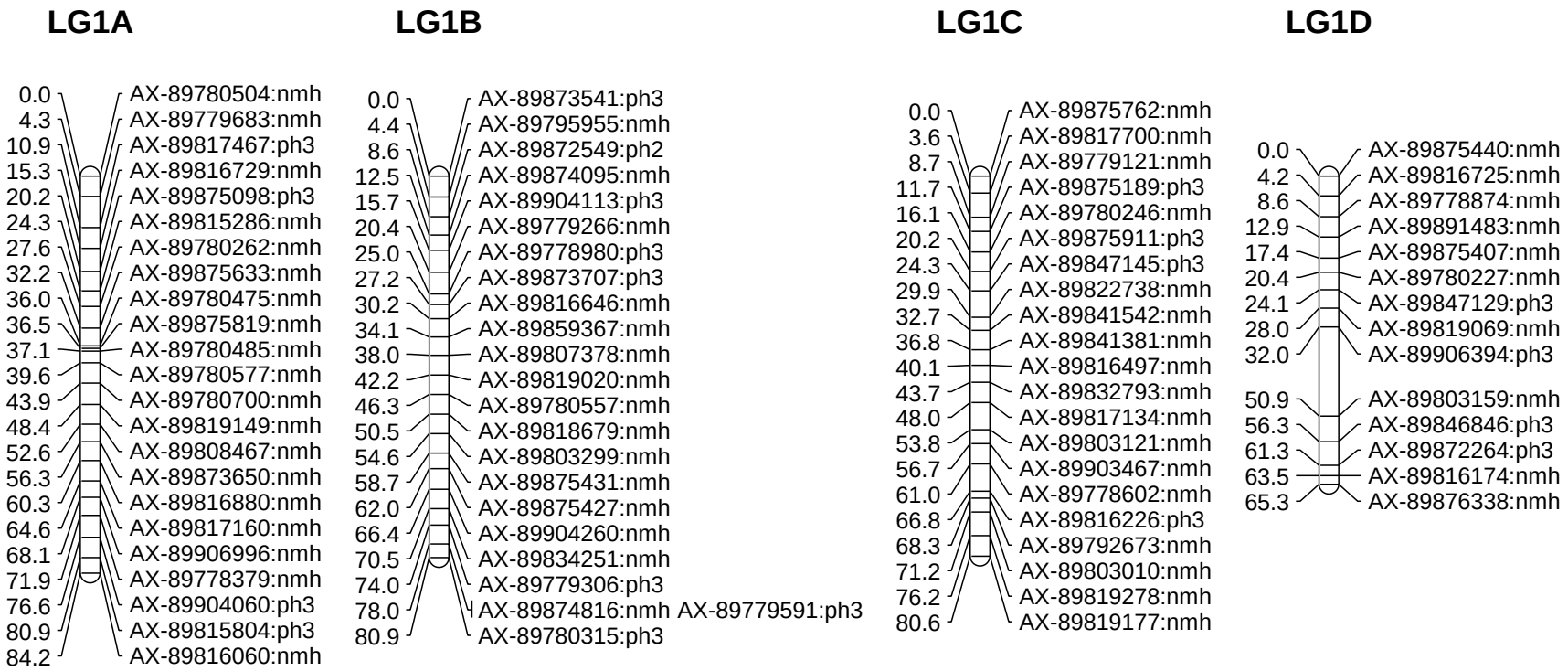

Δ in firmness from Day 1 to Day 7 (2014)
R² = 0.9671
Axis Title
10
5
0
-5
-10
0
10
20
30
40
50
60
70
Genotypes

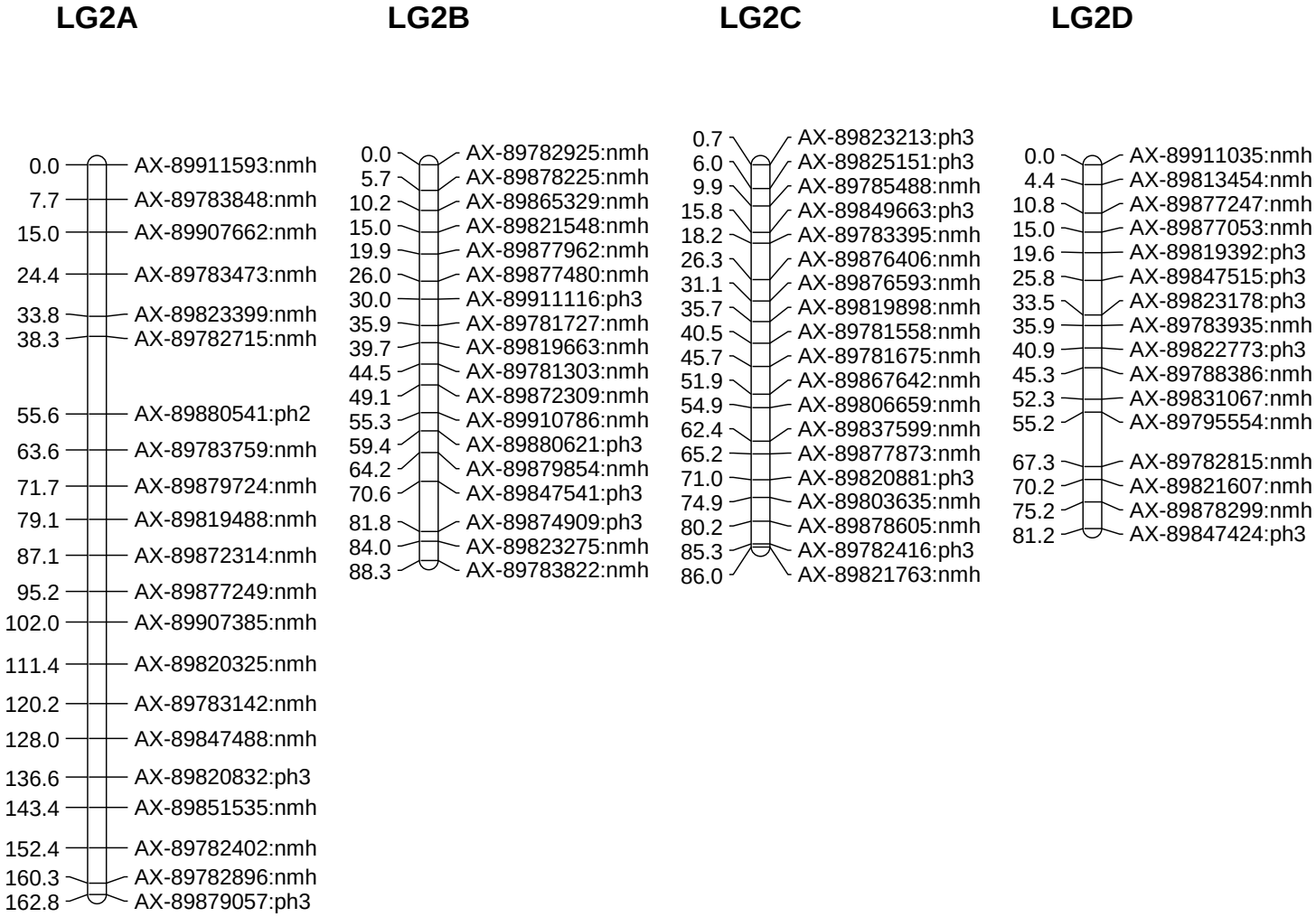

Δ in FW from Day 1 to Day 7 (2014)
R² = 0.7293
Axis Title
3
2
1
0
-1
-2
-3
0
10
20
30
40
50
60
70
Genotypes

Appendix 4.1. Full SNP-based consensus linkage map of an octoploid strawberry mapping progeny (RG×H) composed of 3,933 binned SNP markers, generated by EMR (New Road, East Malling, Kent) with the IStraw90 array. Map spans all 28 linkage groups of *F. ananassa* and a total genetic distance of 2,624.7 cM. The scale in cM is given at the edge of the figure. Retrieved from Antanaviciute, (2016) with a permission.



Appendix 4.2. SNP-based genetic linkage map of the four homoeologous chromosome pairs of the seven homoeologous groups of the (RG×H) population, showing the positions of 523 markers (SNP) distributed over 28 linkage groups (Corresponding to the 56 chromosomes).

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15.0 AX-89907662:nmh
24.4 AX-89783473:nmh
33.8 AX-89823399:nmh
38.3 AX-89782715:nmh
55.6 AX-89880541:ph2
63.6 AX-89783759:nmh
71.7 AX-89879724:nmh
79.1 AX-89819488:nmh
87.1 AX-89872314:nmh
95.2 AX-89877249:nmh
102.0 AX-89907385:nmh
111.4 AX-89820325:nmh
120.2 AX-89783142:nmh
128.0 AX-89847488:nmh
136.6 AX-89820832:ph3
143.4 AX-89851535:nmh
152.4 AX-89782402:nmh
160.3 AX-89782896:nmh
162.8 AX-89879057:ph3
LG2B
0.0 AX-89782925:nmh
5.7 AX-89878225:nmh
10.2 AX-89865329:nmh
15.0 AX-89821548:nmh
19.9 AX-89877962:nmh
26.0 AX-89877480:nmh
30.0 AX-89911116:ph3
35.9 AX-89781727:nmh
39.7 AX-89819663:nmh
44.5 AX-89781303:nmh
49.1 AX-89872309:nmh
55.3 AX-89910786:nmh
59.4 AX-89880621:ph3
64.2 AX-89879854:nmh
70.6 AX-89847541:ph3
81.8 AX-89874909:ph3
84.0 AX-89823275:nmh
88.3 AX-89783822:nmh
LG2C
0.7 AX-89823213:ph3
6.0 AX-89825151:ph3
9.9 AX-89785488:nmh
15.8 AX-89849663:ph3
18.2 AX-89783395:nmh
26.3 AX-89876406:nmh
31.1 AX-89876593:nmh
35.7 AX-89819898:nmh
40.5 AX-89781558:nmh
45.7 AX-89781675:nmh
51.9 AX-89867642:nmh
54.9 AX-89806659:nmh
62.4 AX-89837599:nmh
65.2 AX-89877873:nmh
71.0 AX-89820881:ph3
74.9 AX-89803635:nmh
80.2 AX-89878605:nmh
85.3 AX-89782416:ph3
86.0 AX-89821763:nmh
LG2D
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4.4 AX-89813454:nmh
10.8 AX-89877247:nmh
15.0 AX-89877053:nmh
19.6 AX-89819392:ph3
25.8 AX-89847515:ph3
33.5 AX-89823178:ph3
35.9 AX-89783935:nmh
40.9 AX-89822773:ph3
45.3 AX-89788386:nmh
52.3 AX-89831067:nmh
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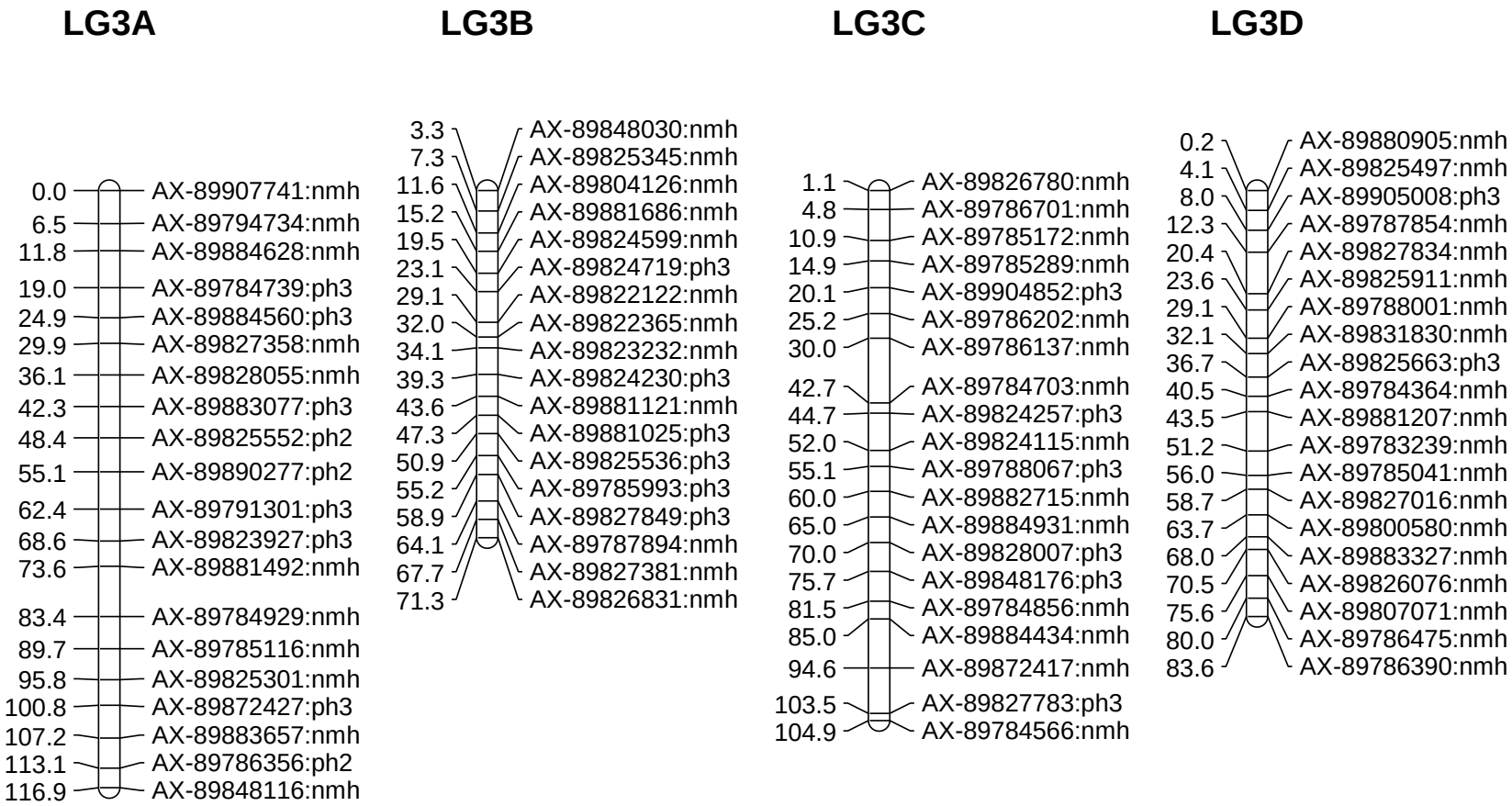
75.2 AX-89878299:nmh
81.2 AX-89847424:ph3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11.8 AX-89884628:nmh
19.0 AX-89784739:ph3
24.9 AX-89884560:ph3
29.9 AX-89827358:nmh
36.1 AX-89828055:nmh
42.3 AX-89883077:ph3
48.4 AX-89825552:ph2
55.1 AX-89890277:ph2
62.4 AX-89791301:ph3
68.6 AX-89823927:ph3
73.6 AX-89881492:nmh
83.4 AX-89784929:nmh
89.7 AX-89785116:nmh
95.8 AX-89825301:nmh
100.8 AX-89872427:ph3
107.2 AX-89883657:nmh
113.1 AX-89786356:ph2
116.9 AX-89848116:nmh
LG3B
3.3 AX-89848030:nmh
7.3 AX-89825345:nmh
11.6 AX-89804126:nmh
15.2 AX-89881686:nmh
19.5 AX-89824599:nmh
23.1 AX-89824719:ph3
29.1 AX-89822122:nmh
32.0 AX-89822365:nmh
34.1 AX-89823232:nmh
39.3 AX-89824230:ph3
43.6 AX-89881121:nmh
47.3 AX-89881025:ph3
50.9 AX-89825536:ph3
55.2 AX-89785993:ph3
58.9 AX-89827849:ph3
64.1 AX-89787894:nmh
67.7 AX-89827381:nmh
71.3 AX-89826831:nmh
LG3C
1.1 AX-89826780:nmh
4.8 AX-89786701:nmh
10.9 AX-89785172:nmh
14.9 AX-89785289:nmh
20.1 AX-89904852:ph3
25.2 AX-89786202:nmh
30.0 AX-89786137:nmh
42.7 AX-89784703:nmh
44.7 AX-89824257:ph3
52.0 AX-89824115:nmh
55.1 AX-89788067:ph3
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81.5 AX-89784856:nmh
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103.5 AX-89827783:ph3
104.9 AX-89784566:nmh
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4.1 AX-89825497:nmh
8.0 AX-89905008:ph3
12.3 AX-89787854:nmh
20.4 AX-89827834:nmh
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58.7 AX-89827016:nmh
63.7 AX-89800580:nmh
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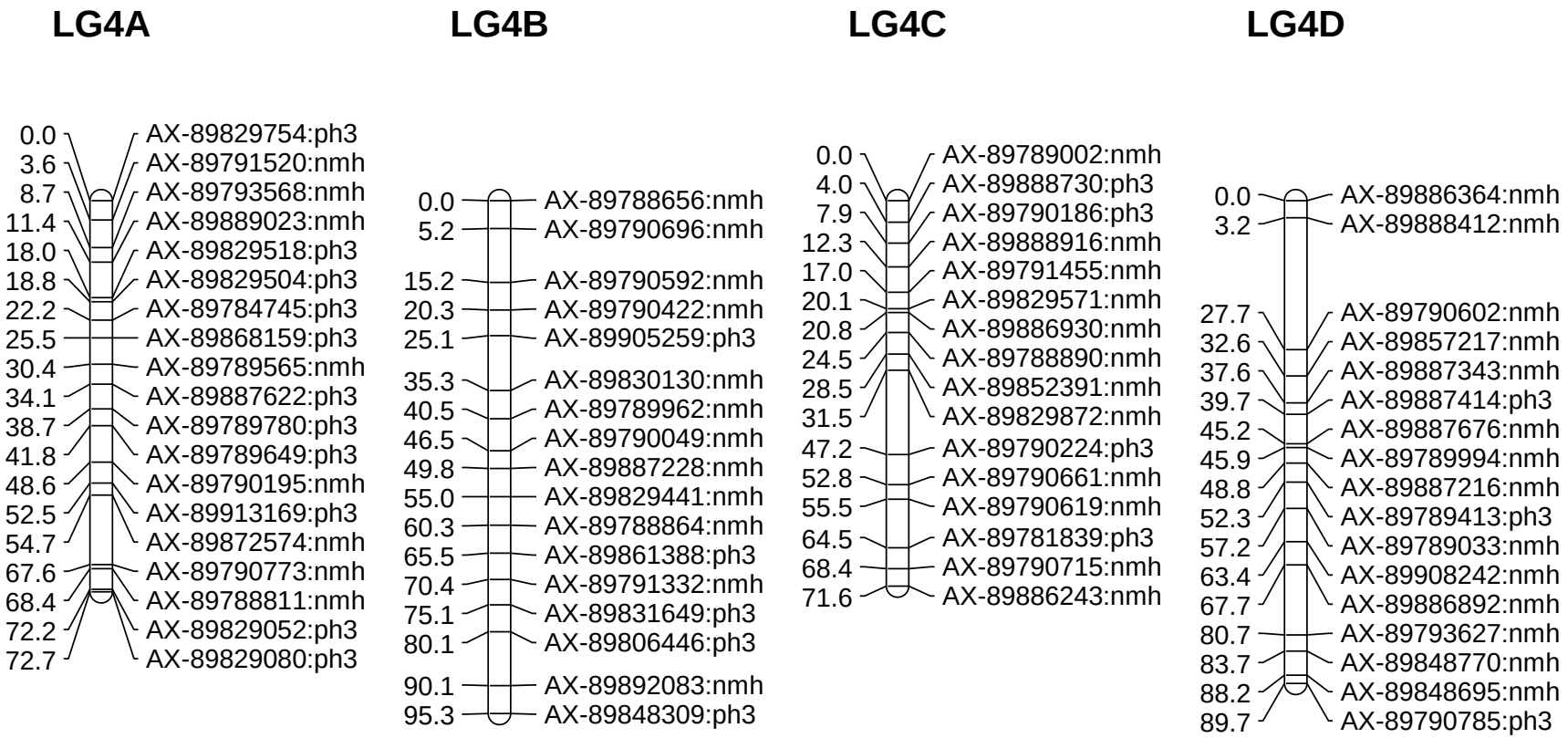
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18.0 AX-89829518:ph3
18.8 AX-89829504:ph3
22.2 AX-89784745:ph3
25.5 AX-89868159:ph3
30.4 AX-89789565:nmh
34.1 AX-89887622:ph3
38.7 AX-89789780:ph3
41.8 AX-89789649:ph3
48.6 AX-89790195:nmh
52.5 AX-89913169:ph3
54.7 AX-89872574:nmh
67.6 AX-89790773:nmh
68.4 AX-89788811:nmh
72.2 AX-89829052:ph3
72.7 AX-89829080:ph3
LG4B
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5.2 AX-89790696:nmh
15.2 AX-89790592:nmh
20.3 AX-89790422:nmh
25.1 AX-89905259:ph3
35.3 AX-89830130:nmh
40.5 AX-89789962:nmh
46.5 AX-89790049:nmh
49.8 AX-89887228:nmh
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60.3 AX-89788864:nmh
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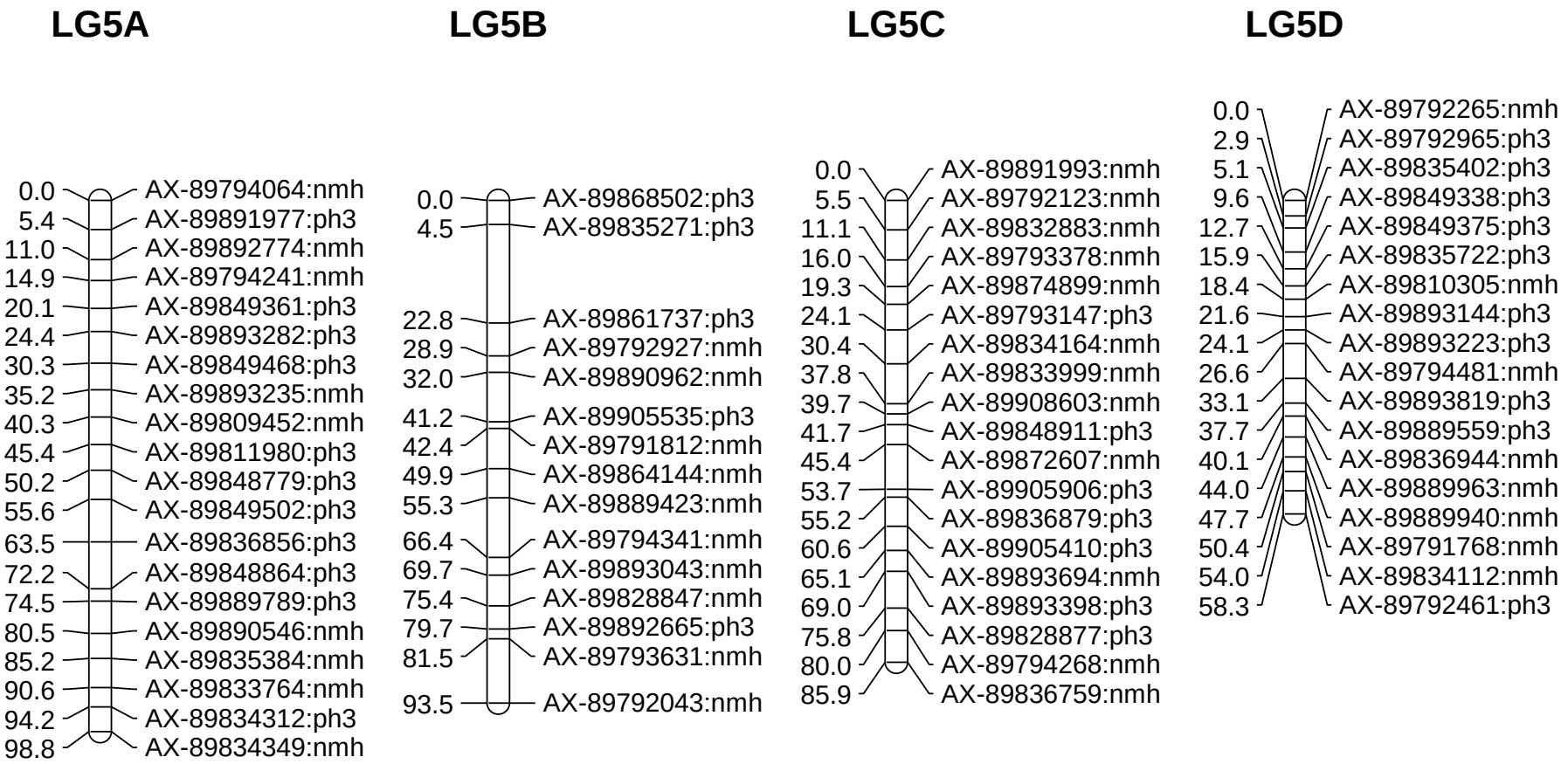
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22.8 AX-89861737:ph3
28.9 AX-89792927:nmh
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41.2 AX-89905535:ph3
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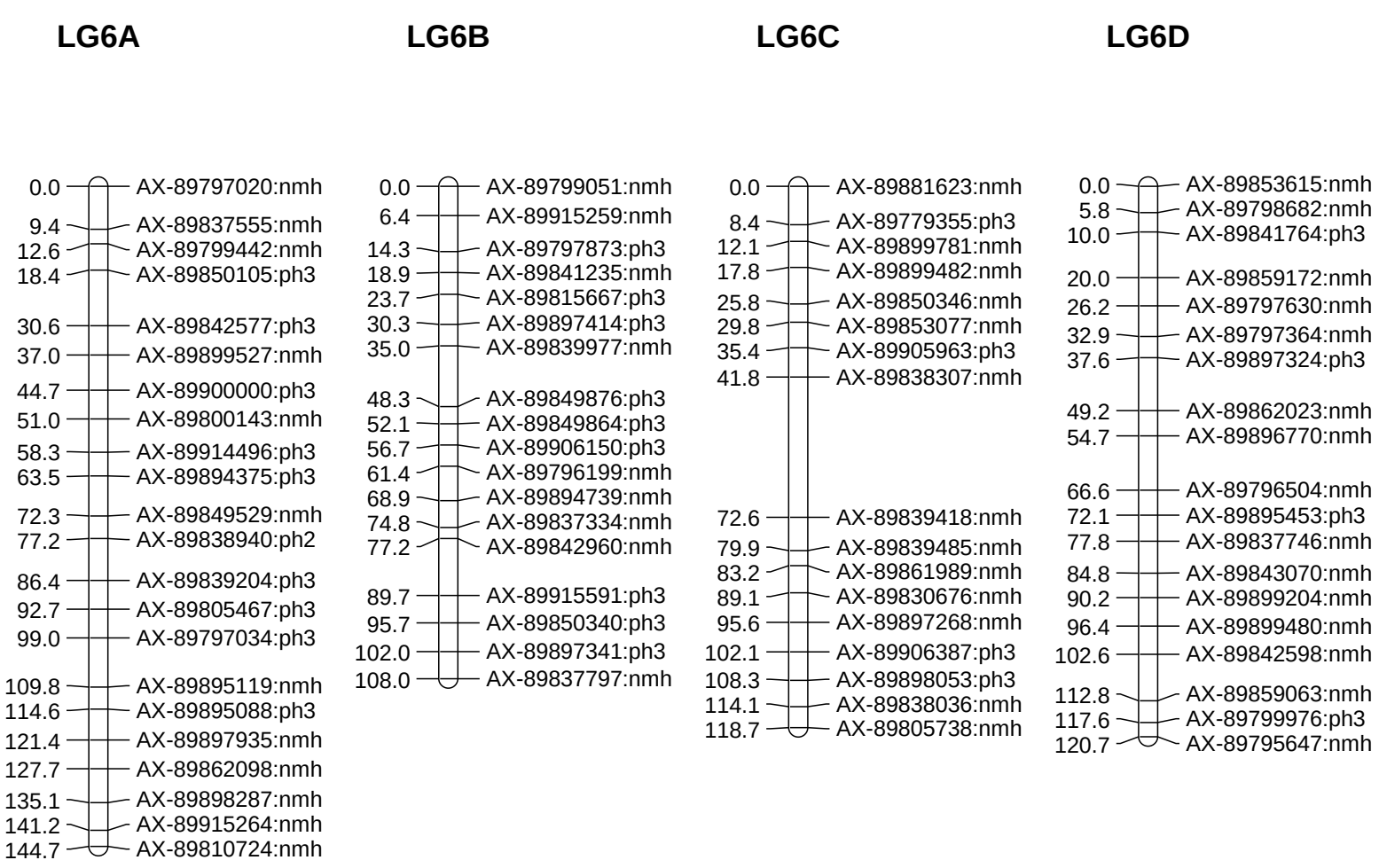
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23.7 AX-89815667:ph3
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74.8 AX-89837334:nmh
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95.7 AX-89850340:ph3
102.0 AX-89897341:ph3
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12.1 AX-89899781:nmh
17.8 AX-89899482:nmh
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37.6 AX-89897324:ph3
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54.7 AX-89896770:nmh
66.6 AX-89796504:nmh
72.1 AX-89895453:ph3
77.8 AX-89837746:nmh
84.8 AX-89843070:nmh
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96.4 AX-89899480:nmh
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117.6 AX-89799976:ph3
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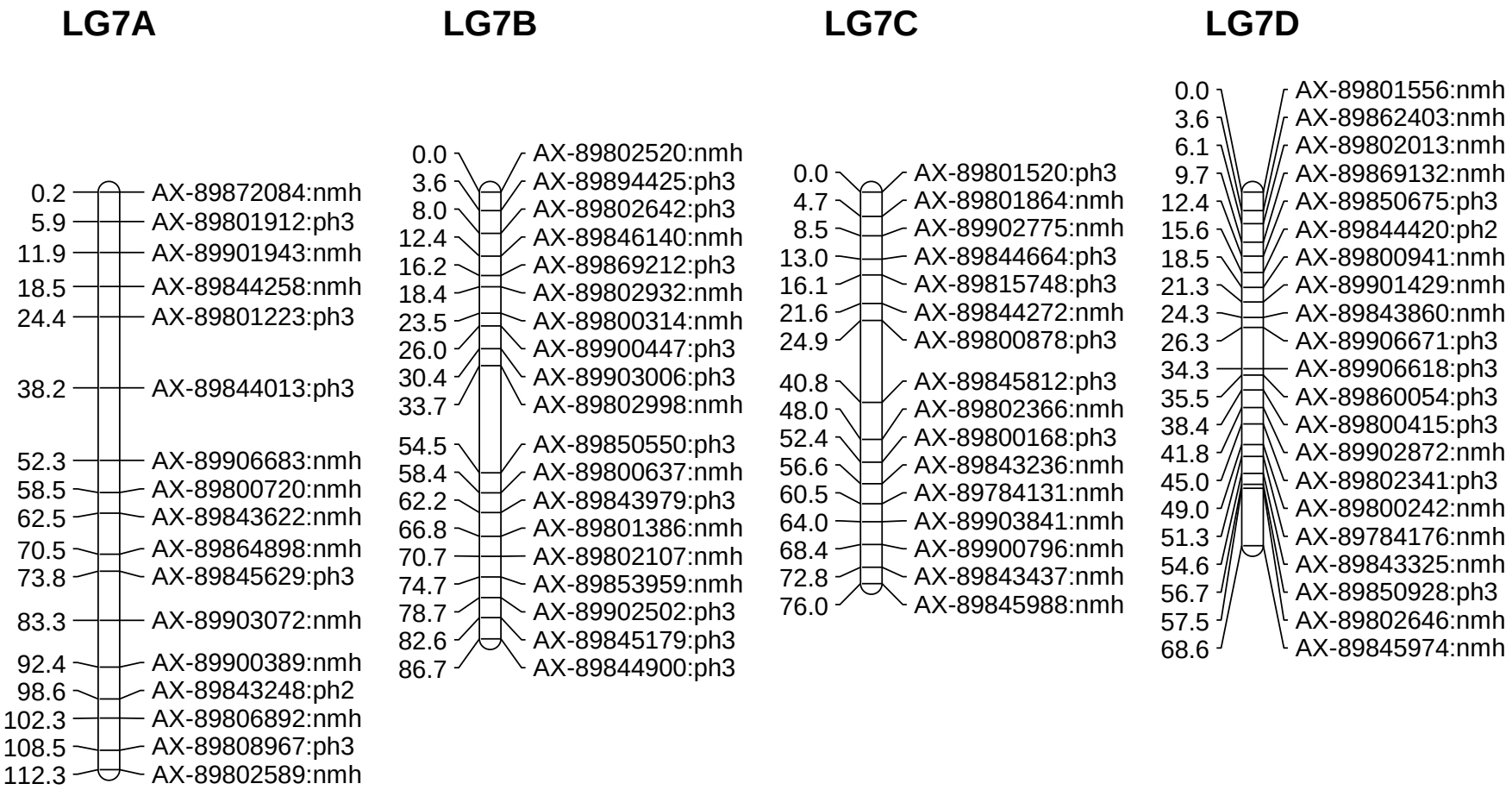
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38.2 AX-89844013:ph3
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62.5 AX-89843622:nmh
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92.4 AX-89900389:nmh
98.6 AX-89843248:ph2
102.3 AX-89806892:nmh
108.5 AX-89808967:ph3
112.3 AX-89802589:nmh
LG7B
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3.6 AX-89894425:ph3
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16.2 AX-89869212:ph3
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23.5 AX-89800314:nmh
26.0 AX-89900447:ph3
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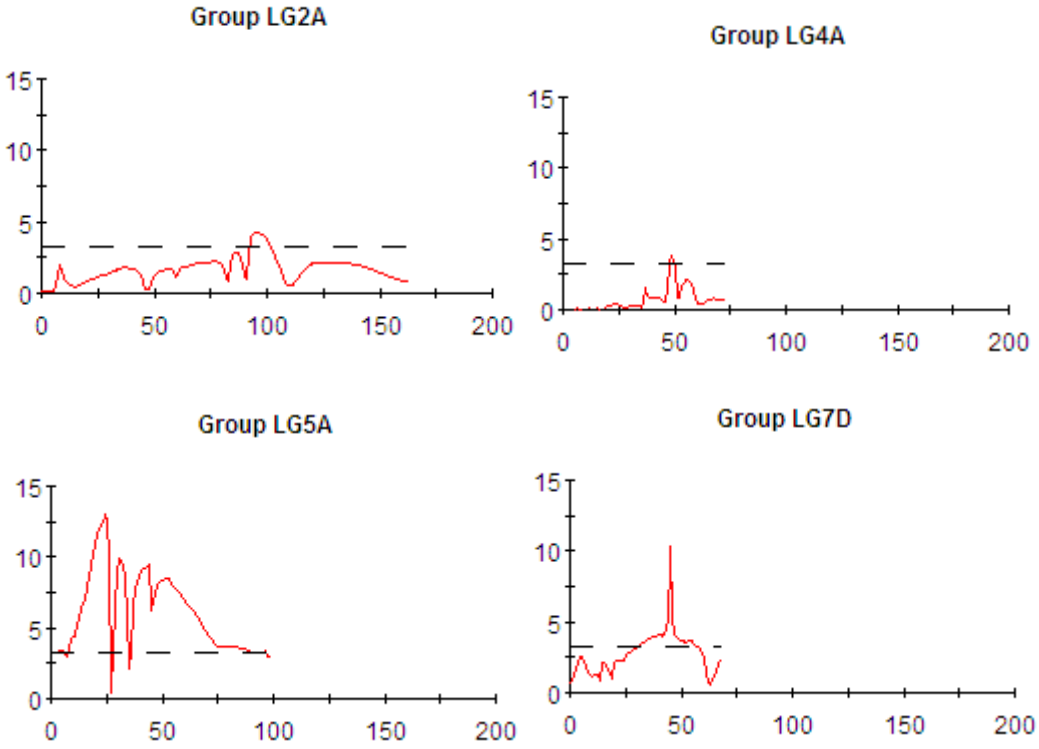
33.7 AX-89802998:nmh
54.5 AX-89850550:ph3
58.4 AX-89800637:nmh
62.2 AX-89843979:ph3
66.8 AX-89801386:nmh
70.7 AX-89802107:nmh
74.7 AX-89853959:nmh
78.7 AX-89902502:ph3
82.6 AX-89845179:ph3
86.7 AX-89844900:ph3
LG7C
0.0 AX-89801520:ph3
4.7 AX-89801864:nmh
8.5 AX-89902775:nmh
13.0 AX-89844664:ph3
16.1 AX-89815748:ph3
21.6 AX-89844272:nmh
24.9 AX-89800878:ph3
40.8 AX-89845812:ph3
48.0 AX-89802366:nmh
52.4 AX-89800168:ph3
56.6 AX-89843236:nmh
60.5 AX-89784131:nmh
64.0 AX-89903841:nmh
68.4 AX-89900796:nmh
72.8 AX-89843437:nmh
76.0 AX-89845988:nmh
LG7D
0.0 AX-89801556:nmh
3.6 AX-89862403:nmh
6.1 AX-89802013:nmh
9.7 AX-89869132:nmh
12.4 AX-89850675:ph3
15.6 AX-89844420:ph2
18.5 AX-89800941:nmh
21.3 AX-89901429:nmh
24.3 AX-89843860:nmh
26.3 AX-89906671:ph3
34.3 AX-89906618:ph3
35.5 AX-89860054:ph3
38.4 AX-89800415:ph3
41.8 AX-89902872:nmh
45.0 AX-89802341:ph3
49.0 AX-89800242:nmh
51.3 AX-89784176:nmh
54.6 AX-89843325:nmh
56.7 AX-89850928:ph3
57.5 AX-89802646:nmh
68.6 AX-89845974:nmh

Appendix 4.3. LOD profiles for TSS-7-14 as an example, TSS values expression (shown in red) on linkage groups have QTL (position is indicated in cM). Horizontal line marks the significant threshold for each QTL.



Appendix 4.4. Table of cofactors.

Trait	QTL analysis	Cofactors		
		LG	Position (cM)	LOD
TSS/TA-1-13	IM	4A	68.55	3.19
		4A	24.918	3.17
		4B	84.185	3.15
	MQM-1	3A	68.553	3.55
		3D	67.988	3.16
		3C	42.689	3
	MQM-2	4B	60.282	4.1
		4D	88.218	3.64
	MQM-3	3A	68.553	4.42
		3C	42.698	3.32
		1A	84.185	3.01
	rMQM	3A	68.553	4.06
L-1-13	IM	4B	70.412	3.52
	MQM-1	4B	70.142	3.52
		6B	6.352	3.27
	MQM-2	4B	70.142	5.26
		6B	6.352	3.27
	rMQM	4B	70.142	5.26
		6B	6.352	3.27
TSS/TA-7-13	IM	4B	60.282	2.7
	MQM-1	3C	42.698	3.65
	MQM-2	4B	60.282	4.54
		4B	52.968	3.21
	MQM-3	4B	60.282	4.54
		3C	42.698	3.56
	rMQM	4B	60.282	4.52
		3C	42.698	3.65
L-7-13	IM	2B	84.043	2.14
		2B	88.275	2.04
	MQM-1	1A	35.979	3.34
		1A	36.524	3.34
		1A	37.129	3.25
		1A	32.217	3.24
		6C	89.125	3.22
	MQM-2	2B	84.043	4.09
		2B	88.275	3.98
		2B	81.884	3.56
	MQM-3	1A	35.979	3.24
		1A	36.524	3.24
	MQM-4	2B	84.043	3.13
		2B	59.42	3.01

Trait	QTL analysis	Cofactors		
		LG	Position (cM)	LOD
	MQM-5	1A	32.217	5.18
		1A	35.979	5.05
		1A	36.524	5.03
		1A	27.646	4.99
		1A	37.129	4.95
		1A	39.557	4.87
	rMQM	1A	37.129	4.52
		2B	59.42	3.34
a-7-13	IM	1A	32.217	4.15
		1A	35.979	3.97
		3C	10.946	3.56
		3C	20.139	6.42
	MQM-1	1A	32.217	3.14
	MQM-2	1A	32.217	4.17
		1A	27.646	3.78
	MQM-3	1A	27.646	3.78
	MQM-4	1A	27.646	3.78
	rMQM	1A	32.217	4.15
Pel-1-14	IM	4B	0	4.38
	MQM-1	4B	0	4.38
	MQM-2	4B	0	4.38
	rMQM	4B	0	4.38
EA-1-14	IM	6C	25.848	3.57
	MQM-1	6C	25.848	3.57
		4D	47.831	3.38
	MQM-2	6C	25.848	4.07
		4D	47.831	3.38
	rMQM	6C	25.848	4.07
		6C	29.824	3.64
		4D	48.831	3.38
		6C	35.42	3.25
TSS/TA-1-14	IM	3D	40.511	2.95
		6A	37.024	2.7
	MQM-1	3A	89.707	3.45
	MQM-2	6A	37.024	4.43
		3D	40.511	3.81
	MQM-3	7B	23.512	3.59
		3A	89.707	3.45
	MQM-4	6A	37.024	5.27
		3D	40.511	4.49
	MQM-5	3A	89.707	5.4
		6A	37.024	4.19
		3D	40.511	3.91
		7B	23.512	3.59

Trait	QTL analysis	Cofactors		
		LG	Position (cM)	LOD
	rMQM	3A	89.707	5.4
		6A	37.024	4.19
		3D	40.511	3.91
		7B	23.512	3.59
TSS-4-14	IM	1A	4.327	3.42
	MQM-1	1A	4.327	3.42
	rMQM	1A	4.327	3.42
TSS/TA-4-14	IM	7A	0.18	4.27
		5B	22.768	3.52
	MQM-1	6A	30.635	4.68
		7A	0.18	3.75
	MQM-2	5B	22.768	4.78
		7A	0.18	4.73
	MQM-3	7A	0.18	5.11
		5B	22.768	4.78
		6A	30.635	4.68
	rMQM	7A	0.18	5.11
		5B	22.768	4.78
		6A	30.635	4.68
FW-7-14	IM	3A	83.392	3.74
	MQM-1	3A	83.392	3.74
		6B	52.054	3.25
	MQM-2	3A	83.392	5.1
		1B	15.703	3.36
		1D	24.052	3.25
		6B	52.054	3.25
	MQM-3	3A	83.392	6.46
		6B	52.054	4.27
		1B	42.196	3.65
	MQM-4	3A	83.392	5.77
		1B	15.703	5.48
		6B	52.054	3.72
	MQM-5	3A	83.392	7.97
		6B	52.054	3.92
		1B	15.703	3.36
	rMQM	3A	83.392	7.97
		6B	52.054	4.09
		1B	15.703	3.26
TA-4-14	IM	2C	54.864	3.73
		6A	37.024	2.61
	MQM-1	2C	54.864	3.73
		6A	37.024	2.48
	rMQM	2C	54.864	3.73
Pel-4-14	IM	2B	81.844	3.76

Trait	QTL analysis	Cofactors		
		LG	Position (cM)	LOD
	MQM-1	2B	81.844	3.76
	MQM-2	2B	81.844	3.76
	rMQM	2B	81.844	3.76
FW-1-14	IM	3A	83.392	3.02
	MQM-1	3A	83.392	3.81
		6B	52.054	3.25
	MQM-2	3A	83.392	3.21
		6B	52.054	3.25
		1B	15.703	3.24
	rMQM	3A	83.392	5.21
		6B	52.054	3.25
		1B	15.703	3.24
TSS-7-14	IM	5A	24.383	3.9
	MQM-1	5A	24.383	3.9
		7D	45.015	3.49
	MQM-2	5A	24.383	6.23
		6C	102.116	4.24
		7A	11.881	3.6
		7D	45.015	3.49
		1C	53.791	3.29
		2A	95.175	3.29
	MQM-3	7D	45.015	9.88
		5A	24.383	9.87
		4A	48.608	3.94
		2A	95.175	3.92
		6C	95.604	6.52
	MQM-4	5A	24.383	12.98
		6C	95.604	7.13
		2A	95.175	4.23
		4A	48.608	3.78
		7D	45.015	10.32
	rMQM	5A	24.383	12.98
		7D	45.015	10.32
		6C	95.175	7.13
		2A	95.175	4.23
		4A	48.608	3.78
TA-7-14	IM	5C	19.34	3.26
		6A	37.024	2.95
		2C	54.864	2.5
	MQM-1	7D	0	3.83
		7D	3.608	3.38
	MQM-2	5C	19.34	4.71
		5C	0	4.13
		2C	54.864	3.27

Trait	QTL analysis	Cofactors		
		LG	Position (cM)	LOD
		3A	116.93	3.24
	MQM-3	7D	0	5.21
		5C	19.34	3.78
		5C	15.985	3.66
	rMQM	5C	19.34	4.5
EA-7-14	IM	6A	99.005	4.1
		6A	109.839	3.44
	MQM-1	6A	99.005	4.1
		4C	64.537	3.16
	MQM-2	6A	99.005	5.44
		2A	38.335	3.24
		4C	64.537	3.16
	MQM-3	6A	99.005	5.89
		4C	64.537	3.43
		2A	38.335	3.24
	rMQM	6A	99.005	5.89
		4C	64.537	3.4
		2A	38.335	3.24
Pel-7-14	IM	7D	18.517	3.32
	MQM-1	7D	18.517	3.32
	rMQM	7D	18.517	3.32
FW-4-14	IM	3A	83.392	3.76
	MQM-1	3A	83.392	3.76
		6B	52.054	3.41
	MQM-2	3A	83.392	5.2
		6B	52.054	3.41
		1B	15.703	3.26
	MQM-3	3A	83.392	7.97
		6B	52.054	4.09
		1B	15.703	3.26
	rMQM	3A	83.392	7.97
		6B	52.054	4.07
		1B	15.703	3.26
L-4-14	IM	6B	89.744	2.43
	MQM-1	6B	89.744	3.25
	rMQM	6B	89.744	3.25
a-4-14	IM	1B	74.025	3.24
	MQM-1	1B	74.025	3.44
	rMQM	1B	74.025	3.42
Firmness-4-14	IM	1D	17.367	2.73
		3A	62.376	2.7
		6C	12.103	2.69
		2A	0	2.66

Trait	QTL analysis	Cofactors		
		LG	Position (cM)	LOD
	MQM-1	1B	66.393	3.23
	MQM-2	6C	12.103	3.68
	MQM-3	6C	17.755	3.78
		1C	0	3.75
	MQM-4	6C	12.103	4.26
		2A	0	3.22
	MQM-5	6C	17.55	4.03
		1C	0	3.76
		1B	66.393	3.56
	MQM-6	6C	12.103	5.13
		2A	0	4.3
	rMQM	6C	12.103	4.1
Cya-1-14	IM	1D	17.367	3.67
		1D	20.354	3.26
	MQM-1	1D	17.367	3.67
		1A	15.33	3.29
	MQM-2	1D	17.367	4.66
		1A	15.33	3.28
	rMQM	1D	17.367	4.66
		1A	15.33	3.28
TSS/TA-7-14	IM	7A	17.367	3.67
		7A	5.917	3.26
	MQM-1	7A	0.18	4.59
	MQM-2	7A	0.18	4.59
	rMQM	7A	0.18	4.59

Appendix 5.1. Selection protocol for F1 progeny individuals

Objectives

To set up a protocol in order to help selecting seven individuals, plus the parental lines, from candidates' progeny for the 3rd year experiment (GC and sensory analysis). As the target is flavour analysis, the selection was based on sugar and acid content (TSS, TA, and TSS/TA ratio). Fruits are abundant in sugars/acids were selected, so that the taste is likely to be distinctive enough to show differences.

The process:

RG126: Biggest TSS increase during shelf life.

RG100 and RG098: Having the lowest TSS across shelf life.

RG169: Having the highest TSS across shelf life.

RG164: Having the lowest TA across shelf life.

RG086 and RG010: Having the highest TA across shelf life.

General notes:

- Fruit quality (flavour) was found in linked with lowering TA and increasing TSS.
- Selection based on the 1st year dataset.
- Max 0.8% TA and Min 7 ºBRIX ⟹ Acceptable flavour.
- The recommended range of the TSS in strawberries is 7-12 ºBRIX, depending on the genotype.
- The minimum result of TSS recorded was 6.5 and the maximum was 12.7 ºBRIX.

Appendix 5.2. Sensory scoring sheet

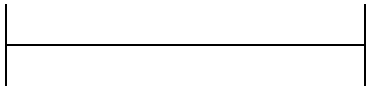
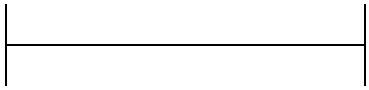
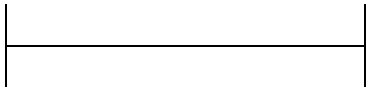
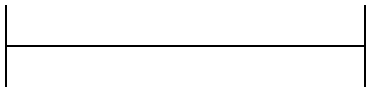
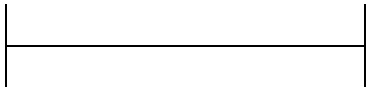
The following scoring sheet was asked of the sensory assessors using compusense 5 software. The assessors must answer questions in order to progress to the next screen.

Control scoring Sheet - Sep. 2015

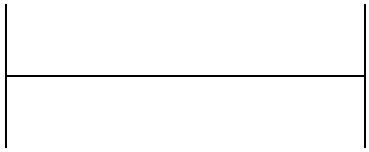
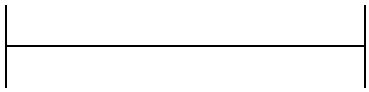
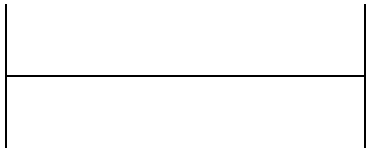
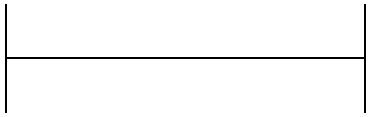
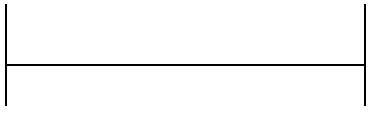
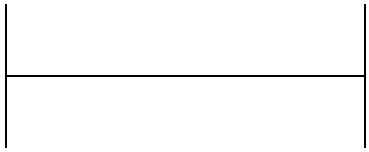
Name:_____ Date:_____ Sample:_____

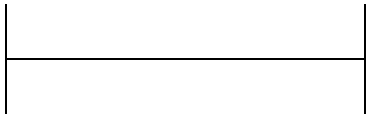
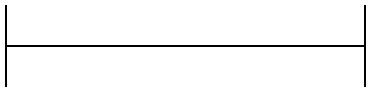
Attribute	Low Anchor Point		High Anchor Point
Odour			
Sweet (candy, sweet)	not		very
Fermented (Lactic acid)	not		very
Zesty (Fresh, citrus)	not		very
Red berry fruit	not		very
Green (Green strawberry)	not		very
Ripeness	not		overripe strawberry
Rubbery	not		very
Off note	not		very

Taste

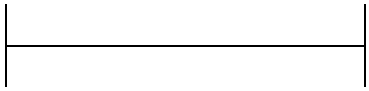
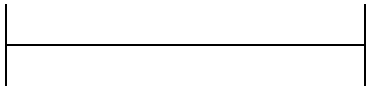
Sweet	not		very
Acid	not		very
Bitter	not		very
Metallic	not		very
Savoury	not		very

Flavour

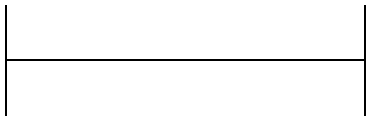
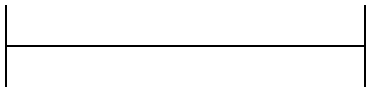
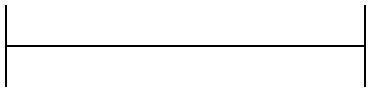
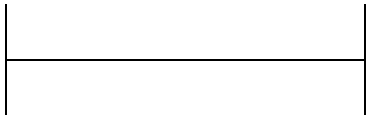
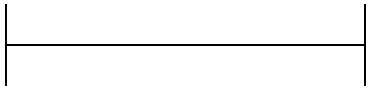
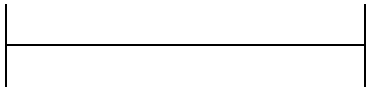
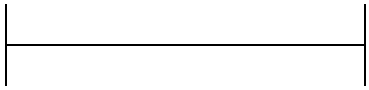
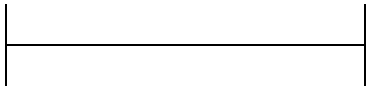
Overall strength of flavour	not		very
Red berry fruit	not		very
Green (Green strawberry and leafy)	not		very
Green (Kiwi and aromatic)	not		very
Ripeness	not		overripe strawberry
Floral (perfume, rosey)	not		very

Cardboard (stale)	not		very
Woody	not		very

Mouth sensation

Fizzy	not		very
Mouthdrying	not		very

Aftertaste

Length of finish	short		long
Acid	not		very
Savoury	not		very
Cardboard (stale)	not		very
Metallic	not		very
Astringent	not		very
Mouthdrying	not		very
Salivating	not		very

Appendix 5.3. Definitions for sensory attributes associated with strawberry

Attribute	Agreed definition	Definition
Odour	Sweet (candy, sweet)	A pleasant, sugary and/or aroma
	Fermented (Lactic acid)	Aroma associated with lactic acid as a result of the fermentation process
	Zesty (Fresh, citrus)	Smell that gives a sharp sensation
	Red berry fruit	Aroma associated with berry fruits
	Green (Green strawberry)	Aroma associated with cut grass and freshness
	Ripeness	Aroma associated with ripe fruits
	Rubbery	Resembling or suggestive of rubbery gloves
	Off note	Aroma associated with deterioration or contamination
Taste	Sweet	Pleasant taste associated with sugar food
	Acid	Acidic sensation associated with sour food
	Bitter	Unpleasant or pungent taste
	Metallic	Having an acrid quality like that of metal
	Savoury	Sharp, taste associated with slightly salty food
Flavour	Overall strength of flavour	Flavour associated with berries
	Red berry fruit	Flavour associated with berries
	Green (Green strawberry and leafy)	Flavour associated with cut grass of freshness
	Green (Kiwi and aromatic)	Flavour associated with cut grass of freshness
	Ripeness	Flavour associated with ripe fruits
	Floral (perfume, rosey)	Flavour associated with perfume or rose
	Cardboard (stale)	Flavour associated with cardboard; rank, unpleasant, and stale: a rancid taste
	Woody	Flavour associated with wood

Attribute	Agreed definition	Definition
Mouth sensation	Fizzy	Associated with fizzy drinks (i.e. carbonated drinks)
	Mouthdrying	The sensation of dryness
After effects	Length of finish	Persistence of the flavour of the sample
	Acid	Persistence of the sour flavour
	Savoury	Persistence of a sharp "salty" flavour upon the tongue
	Cardboard (stale)	Persistence of rank, unpleasant, and stale: a rancid taste
	Metallic	Persistence of an acrid quality like that of metal
	Astringent	Persistence of the sensation of shrinkage of the tongue and soft palate
	Mouthdrying	Persistence of the sensation of dryness
	Salivating	Persistence of the production of saliva within the mouth after swallowing

Appendix 5.4. Factor loading for the combined data (sensory and physiology)

Observs.	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17
O1	0.491	-0.491	0.294	-0.170	-0.253	-0.115	-0.438	-0.110	-0.259	-0.059	-0.005	0.127	-0.118	0.051	-0.087	-0.067	-0.081
O2	0.313	0.081	0.518	-0.087	0.658	0.042	0.151	0.196	-0.219	0.165	0.055	-0.035	0.076	0.187	-0.024	-0.014	-0.043
O3	-0.245	0.593	-0.027	-0.110	-0.170	0.516	-0.024	0.397	-0.137	-0.021	0.047	0.087	0.146	0.212	-0.121	-0.103	0.030
O4	0.391	-0.557	0.476	0.107	-0.320	-0.164	-0.069	0.006	-0.015	0.090	0.207	0.289	0.104	0.072	0.033	0.097	0.058
O5	-0.458	0.562	-0.179	0.331	-0.159	0.357	-0.078	-0.150	-0.052	0.058	0.190	-0.039	-0.220	0.129	-0.146	0.141	0.027
O6	0.626	-0.367	0.467	-0.160	-0.138	-0.321	0.144	0.000	-0.002	-0.154	0.018	-0.147	0.074	0.053	-0.053	-0.079	0.149
O7	-0.271	0.248	-0.405	0.175	-0.344	-0.275	0.454	-0.135	-0.185	0.423	-0.125	0.021	0.007	0.104	0.083	-0.065	0.033
O8	0.274	0.094	0.503	0.241	0.250	0.286	0.497	-0.358	-0.058	-0.158	-0.031	0.117	-0.051	-0.161	-0.095	-0.078	0.012
T1	0.820	0.393	0.019	-0.083	-0.125	0.213	0.046	0.158	0.006	-0.049	-0.096	-0.144	0.157	-0.102	0.077	0.028	-0.057
T2	-0.576	0.198	0.246	0.070	-0.077	-0.494	-0.055	0.332	-0.170	-0.101	-0.333	-0.072	-0.187	0.075	-0.067	-0.013	-0.013
T3	-0.675	0.526	0.140	0.029	0.074	-0.030	-0.005	0.148	-0.201	0.108	-0.027	0.322	0.086	-0.204	0.111	0.010	-0.016
T4	-0.544	0.269	-0.134	-0.381	-0.371	-0.237	0.337	0.023	0.184	-0.067	0.250	0.026	-0.198	-0.013	0.009	-0.121	-0.077
T5	-0.347	-0.683	0.296	0.146	-0.099	0.191	0.218	0.178	-0.367	0.158	0.004	-0.073	-0.058	-0.022	0.025	-0.005	-0.101
F1	0.727	0.441	0.183	0.069	-0.380	0.033	-0.060	0.195	-0.101	-0.003	0.097	0.065	-0.026	-0.167	-0.001	0.017	0.003
F2	0.757	0.538	0.196	-0.034	-0.256	-0.020	-0.020	0.122	0.075	-0.060	0.025	0.048	0.062	-0.010	0.016	-0.011	-0.023
F3	-0.806	0.049	0.037	0.227	-0.251	-0.001	-0.387	-0.055	0.068	-0.189	-0.032	-0.090	0.141	-0.013	0.006	-0.056	-0.087
F4	-0.218	0.276	0.202	0.670	-0.031	-0.077	-0.012	0.019	-0.331	-0.243	0.349	-0.221	-0.030	-0.008	0.190	-0.037	0.046
F5	0.865	0.238	0.119	0.143	-0.174	0.190	0.009	-0.048	0.163	0.154	-0.034	-0.089	-0.081	0.131	0.071	0.052	-0.020
F6	0.725	0.098	0.199	-0.066	-0.320	0.082	0.343	-0.127	-0.154	-0.251	-0.253	-0.008	-0.040	0.078	-0.038	0.114	-0.066
F7	-0.648	-0.490	0.344	0.066	-0.119	0.272	0.065	-0.266	0.097	-0.037	-0.127	0.039	0.007	0.097	0.119	-0.041	-0.022
F8	-0.126	0.237	0.628	-0.560	0.042	0.030	-0.015	-0.095	0.153	0.236	0.305	-0.176	-0.021	-0.023	-0.006	-0.015	-0.065
M1	-0.543	-0.039	0.330	0.178	0.252	-0.033	0.227	0.409	0.405	-0.224	0.022	0.158	-0.160	0.086	0.066	0.049	0.003
M2	-0.600	0.462	0.388	-0.258	-0.086	0.261	-0.181	-0.129	-0.122	0.036	-0.100	0.034	-0.151	-0.099	-0.091	-0.047	0.114
A1	0.503	0.711	0.226	0.178	-0.127	-0.110	-0.017	-0.190	0.173	-0.027	-0.027	0.144	0.015	0.159	0.098	-0.072	-0.032
A2	-0.801	0.352	0.155	0.078	0.075	-0.104	-0.085	-0.291	0.026	-0.082	-0.060	0.021	0.246	0.149	-0.029	-0.019	-0.011

Observs.	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17
A3	-0.449	-0.418	0.447	-0.085	-0.369	0.332	-0.102	0.171	0.171	0.170	-0.185	-0.104	-0.025	-0.016	0.138	-0.002	0.086
A4	-0.610	-0.629	0.148	0.298	-0.208	0.205	0.076	-0.005	0.087	0.017	0.089	0.038	0.058	-0.011	-0.074	0.012	-0.058
A5	-0.749	-0.027	0.006	-0.119	-0.347	-0.114	0.404	0.109	0.041	-0.082	0.074	-0.085	0.243	-0.061	-0.159	0.083	0.009
A6	-0.491	0.412	0.523	-0.030	0.029	-0.497	-0.107	-0.137	-0.046	0.122	0.031	-0.017	0.022	0.001	-0.032	0.131	-0.007
A7	-0.622	0.469	0.181	-0.477	0.060	0.154	0.032	-0.125	-0.113	-0.132	-0.143	-0.066	0.000	-0.016	0.164	0.082	-0.001
A8	0.158	0.348	0.412	0.663	0.038	-0.088	-0.065	0.057	0.288	0.249	-0.180	-0.110	0.017	-0.135	-0.132	-0.050	-0.031
a1	0.072	0.383	0.198	0.034	-0.407	0.057	-0.121	0.429	-0.244	0.063	-0.380	0.263	-0.116	-0.107	0.225	0.292	0.045
a2	0.477	-0.241	0.291	0.201	0.139	-0.325	-0.015	-0.368	0.112	-0.224	-0.097	0.068	0.052	0.302	-0.134	-0.241	-0.285
a3	-0.017	-0.663	0.090	0.316	0.129	-0.306	-0.118	-0.276	-0.103	-0.164	0.119	0.233	-0.085	0.202	-0.076	-0.112	-0.279
a4	0.071	0.587	-0.277	0.108	-0.270	0.512	-0.294	0.304	-0.139	0.076	-0.054	-0.003	-0.018	-0.127	0.050	0.033	0.017
ald1	-0.244	0.005	0.209	-0.439	0.075	0.231	-0.214	-0.095	-0.172	0.315	-0.166	-0.526	-0.270	0.015	-0.054	0.200	-0.203
ald2	-0.029	-0.344	-0.243	-0.084	0.503	0.171	-0.308	0.031	-0.004	0.046	0.132	-0.241	-0.464	0.186	0.194	0.064	0.262
ald3	0.596	0.048	0.201	0.040	0.106	0.344	0.033	0.408	0.147	-0.074	-0.130	0.437	0.105	-0.040	0.119	-0.137	0.152
ald4	-0.049	-0.859	0.171	0.083	0.269	0.063	0.146	-0.144	0.149	-0.130	-0.090	-0.047	-0.035	0.103	0.180	-0.102	0.036
ald5	0.022	-0.298	0.650	0.043	0.149	-0.064	0.157	-0.447	0.020	0.074	-0.268	-0.151	0.144	0.253	-0.163	-0.094	-0.122
ald6	0.404	0.145	-0.076	-0.080	-0.015	0.509	0.027	0.581	0.020	-0.014	0.061	0.256	-0.008	-0.150	-0.043	-0.293	0.162
ald7	0.369	-0.055	-0.063	-0.043	0.156	0.102	0.116	0.518	-0.198	-0.185	0.222	-0.250	-0.374	-0.333	-0.244	-0.113	0.173
ald8	0.179	0.152	-0.241	-0.147	0.030	0.354	-0.044	0.280	0.287	-0.128	-0.003	0.533	0.158	-0.184	0.121	-0.385	0.230
ald9	-0.356	-0.668	0.146	0.322	0.177	0.119	0.133	-0.345	0.213	-0.083	0.168	0.175	-0.059	-0.035	-0.038	-0.044	-0.035
f1	0.075	0.185	0.135	0.439	0.047	-0.060	-0.288	-0.001	0.295	-0.096	0.406	0.152	0.327	0.059	-0.268	-0.313	-0.309
f2	0.137	0.071	0.014	-0.008	0.218	-0.061	-0.004	0.190	0.546	-0.307	0.375	0.394	-0.364	0.153	0.123	-0.118	-0.124
e1	0.135	0.125	-0.318	0.169	0.264	0.178	0.058	0.406	-0.122	-0.050	0.480	-0.293	-0.359	-0.109	-0.040	0.246	0.171
e2	0.186	0.106	0.144	-0.164	0.093	-0.294	0.248	0.139	0.271	-0.146	0.482	0.013	-0.230	0.020	0.241	0.241	-0.483
e3	0.014	0.417	0.158	0.268	0.372	-0.372	-0.007	-0.002	0.101	0.437	0.333	-0.057	0.179	-0.075	-0.234	-0.109	0.183
e4	-0.084	0.148	-0.205	-0.106	0.255	0.281	-0.018	0.181	0.004	-0.111	0.462	-0.127	-0.166	0.257	0.354	0.083	0.522
e5	0.167	0.087	0.385	-0.128	0.199	-0.589	0.046	-0.083	0.189	0.200	0.296	0.065	-0.102	0.273	-0.176	0.078	-0.338
e6	0.210	0.326	0.088	0.022	-0.354	-0.324	-0.117	-0.207	0.029	0.238	-0.252	0.060	0.494	0.118	-0.128	-0.236	-0.317

Observs.	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17
e7	0.146	0.200	0.311	-0.290	-0.001	-0.356	0.210	-0.179	0.023	0.076	0.288	-0.178	0.211	0.291	-0.153	0.352	-0.397
e8	0.389	-0.102	0.172	-0.037	0.116	-0.046	0.076	0.335	-0.300	0.058	0.388	-0.174	-0.329	0.034	-0.008	0.414	0.345
e9	-0.088	-0.091	0.477	-0.077	0.152	-0.397	-0.003	-0.557	-0.031	0.282	-0.047	-0.180	0.224	0.296	-0.045	-0.003	0.070
e10	0.417	0.015	0.521	0.080	0.013	-0.234	0.008	-0.333	-0.011	-0.203	-0.236	-0.077	0.160	0.295	-0.184	-0.188	-0.311
e11	0.207	0.252	0.224	0.150	-0.128	-0.499	-0.376	0.040	0.015	-0.222	0.016	0.269	-0.035	0.262	0.007	-0.173	-0.436
e12	0.125	0.336	-0.050	-0.131	-0.160	-0.297	-0.031	-0.139	0.105	0.186	-0.236	0.040	0.632	-0.078	-0.198	-0.333	-0.245
e13	0.284	0.115	-0.315	0.018	-0.504	-0.220	-0.064	-0.261	0.182	0.061	-0.119	0.149	0.163	0.248	-0.074	-0.493	-0.151
e14	0.054	0.111	0.290	-0.386	-0.116	-0.432	0.179	-0.011	0.078	0.042	0.309	-0.112	0.055	0.238	-0.020	0.369	-0.456
e15	0.629	-0.075	0.178	0.278	0.126	-0.067	0.106	0.225	-0.176	0.015	0.362	-0.246	-0.272	-0.242	0.163	0.101	-0.127
e16	0.403	0.017	0.466	0.085	-0.120	-0.089	0.070	0.144	-0.273	0.201	0.091	0.019	-0.018	0.100	0.394	0.526	-0.019
e17	0.514	-0.008	0.031	0.222	0.199	-0.131	0.048	0.228	-0.023	-0.232	0.278	-0.189	-0.531	-0.299	-0.050	-0.182	-0.033
e18	-0.001	-0.066	-0.002	0.217	-0.139	-0.128	0.050	-0.521	0.234	-0.272	-0.268	0.237	0.449	-0.241	-0.046	-0.311	-0.150
e19	0.272	0.194	0.229	-0.142	0.229	-0.321	0.266	-0.102	0.221	0.076	0.478	0.342	0.084	0.010	-0.240	0.263	-0.213
e20	0.321	0.010	0.429	-0.084	-0.004	-0.365	-0.081	-0.432	0.110	-0.060	-0.362	0.109	0.231	0.264	-0.185	-0.088	-0.238
e21	0.515	-0.098	0.415	0.195	0.225	-0.118	0.093	-0.317	0.189	-0.284	-0.209	0.116	-0.062	0.150	0.016	-0.279	-0.252
e22	0.626	0.246	0.036	0.389	-0.128	0.105	-0.049	0.276	0.079	0.141	-0.001	0.190	-0.112	-0.246	0.318	-0.094	-0.206
e23	0.639	-0.232	0.094	0.521	0.000	0.006	0.196	0.053	-0.265	-0.026	0.026	-0.069	-0.257	-0.088	0.185	0.042	-0.174
e24	0.159	-0.010	0.474	-0.092	-0.034	-0.321	0.092	-0.524	0.089	-0.130	-0.075	0.324	0.191	-0.017	-0.385	-0.135	-0.125
e25	0.450	-0.216	0.489	0.163	0.318	-0.262	0.092	-0.220	0.253	0.016	0.047	0.135	-0.197	0.233	-0.053	-0.061	-0.273
e26	0.255	-0.085	0.346	0.015	-0.169	0.065	0.016	-0.383	0.078	-0.295	-0.562	-0.069	0.215	0.247	-0.037	-0.266	-0.186
e27	-0.130	-0.467	0.267	0.451	0.033	-0.084	0.271	-0.294	0.254	-0.245	-0.164	0.069	0.127	0.306	-0.126	-0.176	-0.095
e28	0.271	-0.187	0.096	0.295	-0.089	-0.431	-0.440	-0.014	0.231	-0.089	-0.049	0.419	-0.144	0.250	0.019	-0.204	-0.208
e29	0.545	-0.177	0.216	0.332	-0.035	0.063	-0.058	-0.372	-0.065	-0.388	-0.096	-0.173	0.142	0.113	-0.184	-0.281	-0.170
e30	0.493	0.032	-0.174	0.186	0.055	0.272	0.077	0.200	0.071	0.034	0.293	-0.364	-0.435	-0.282	0.148	-0.064	0.220
e31	0.444	0.403	-0.170	0.116	0.186	0.263	0.071	0.322	0.296	0.133	0.301	-0.148	-0.319	-0.078	0.205	-0.132	-0.010
k2	0.302	-0.057	0.442	0.085	0.157	-0.426	-0.199	-0.187	0.288	0.241	0.059	0.122	-0.175	0.314	-0.179	-0.220	-0.230
k3	-0.087	0.190	0.327	-0.327	0.093	-0.124	0.120	-0.212	0.073	-0.438	-0.551	0.063	-0.116	-0.287	0.196	0.115	0.087

Observs.	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17
t1	0.353	0.301	-0.426	0.265	-0.529	-0.012	0.070	0.112	-0.281	0.271	-0.111	0.035	0.180	-0.062	0.023	-0.182	-0.014
t2	0.293	0.458	-0.388	0.149	-0.550	0.030	0.051	0.113	-0.228	0.171	0.091	0.092	0.211	0.109	0.127	-0.193	0.067
t3	-0.350	0.432	0.114	-0.530	-0.219	-0.173	-0.171	-0.113	0.149	-0.112	-0.275	0.004	0.067	-0.202	0.165	0.122	0.282
t4	0.339	0.286	-0.445	0.273	-0.534	0.000	0.070	0.079	-0.268	0.279	-0.104	0.028	0.185	-0.054	0.012	-0.189	-0.018
t5	0.336	0.245	-0.395	0.359	-0.476	0.054	-0.057	-0.034	-0.311	0.174	0.040	-0.062	0.339	-0.049	-0.013	-0.226	-0.085
t6	0.438	0.273	-0.356	0.323	-0.509	0.007	0.025	0.057	-0.325	0.219	-0.040	0.030	0.203	-0.092	0.013	-0.174	-0.061
t7	0.358	0.280	-0.384	0.340	-0.461	0.000	-0.084	0.043	-0.336	0.112	0.049	-0.033	0.291	-0.103	-0.056	-0.253	-0.131
t8	0.446	0.073	-0.309	0.397	-0.379	0.017	-0.105	0.034	-0.318	0.150	0.255	-0.045	0.306	-0.193	-0.066	-0.201	-0.143
c1	-0.059	0.213	0.060	-0.224	0.415	0.091	0.191	0.261	0.295	-0.186	0.568	0.152	-0.215	0.185	0.189	0.147	0.092
c2	0.049	0.225	0.405	-0.301	0.152	-0.281	0.239	-0.281	0.285	-0.017	0.417	-0.084	0.084	0.265	-0.107	0.113	-0.299
c3	-0.374	-0.115	-0.187	-0.119	0.340	0.073	0.057	0.032	0.325	-0.497	0.110	0.312	0.021	-0.372	0.040	-0.046	0.259
c4	-0.294	0.008	-0.071	-0.072	0.086	-0.175	0.016	-0.141	0.232	-0.430	-0.330	0.260	0.459	-0.378	-0.147	-0.227	-0.054
c5	-0.447	-0.383	-0.304	-0.187	0.285	0.430	0.032	0.057	-0.159	0.144	-0.079	0.248	0.013	0.128	-0.136	0.027	-0.326
EA	0.208	0.158	0.551	0.017	-0.176	0.016	-0.083	-0.068	0.488	-0.039	-0.146	0.200	-0.351	-0.079	-0.136	-0.350	0.118
Pel	0.049	0.569	-0.225	0.183	-0.188	0.271	0.267	0.019	0.081	0.314	0.402	0.259	-0.101	-0.071	0.201	0.050	0.132
Cya	-0.368	0.052	0.440	-0.053	-0.132	0.253	0.289	-0.078	0.227	-0.252	-0.163	0.185	-0.274	-0.284	-0.017	0.135	0.380
FW	-0.161	-0.224	-0.310	-0.196	0.412	0.148	-0.139	-0.015	-0.355	0.228	0.317	-0.206	-0.128	0.401	0.252	0.067	0.115
Firmness	0.417	-0.483	-0.209	0.487	0.338	0.018	0.074	0.002	-0.089	0.045	0.266	-0.016	-0.060	-0.157	-0.067	-0.147	-0.239
TSS	0.572	0.507	0.010	-0.117	-0.125	0.216	-0.200	0.246	0.142	0.180	0.208	-0.003	-0.200	0.095	0.263	-0.132	-0.086
TA	0.574	0.004	-0.239	-0.113	-0.205	0.158	0.398	0.354	0.184	-0.109	0.300	-0.021	-0.061	0.067	-0.277	-0.169	0.009
TSS/TA%	0.180	0.439	0.146	-0.006	0.065	0.051	-0.528	0.037	-0.089	0.286	-0.020	0.040	-0.073	0.166	0.576	0.056	-0.085
L*	0.226	-0.237	-0.035	0.259	-0.083	0.078	0.140	-0.177	0.172	0.100	-0.174	0.014	0.344	0.171	-0.199	-0.600	-0.378
a*	0.013	-0.563	0.386	0.466	0.311	-0.094	0.179	-0.177	0.096	-0.205	-0.105	-0.130	-0.147	-0.169	-0.008	-0.095	-0.100
b*	0.097	-0.635	0.260	0.376	0.299	-0.235	0.138	-0.195	-0.004	-0.240	-0.111	-0.113	-0.220	0.039	-0.196	-0.021	-0.070

The results corresponding to the supplementary variables are displayed in the second part of the table

Supporting evidence

Conferences:

Conference attended	Presentation (oral/poster)	Date
Fruits and Roots: A celebration and Forward Look	Attendee	6-7/11/2013
PhD School Conference (NGR conference), 2014	Poster titled: “Phenotyping of strawberry quality and nutritional traits of “Redgauntlet x Hapil” progeny”	Feb, 2014
Breeding Plants for the Future	Poster titled: “Phenotyping of strawberry quality and nutritional traits of “Redgauntlet x Hapil” progeny”	15/05/2014
Graduate conference (UoR), 2014	Poster titled: “How to improve strawberry nutritional and quality traits?”	July, 2014
7th European Short Course on Quality and Safety of Fresh-cut Produce	Poster titled: “Phenotyping of strawberry quality and nutritional traits of “Redgauntlet x Hapil” progeny”	21-23/01/2015
PhD School Conference (NGR conference), 2015	Digital poster presentation titled: “Phenotyping of fruit quality traits in octoploid strawberry (Fragaria x ananassa)”	Feb, 2015
Workshop on Latest technologies for crop improvement	Poster titled: “Phenotyping of fruit quality traits in octoploidstrawberry (Fragariax ananassa)”	Feb, 2015
Eucarpia General Congress 2016 "Plant Breeding: The Art of Bringing Science to Life"	Paper titled: “QTL identification and phenotyping of strawberry fruit of an octoploid population for quality traits”	Aug, 2016

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Mapping QTL underlying fruit quality traits in an F1 strawberry population

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Strawberry is a highly perishable fruit and improving its nutritional and quality traits is a fundamental goal for breeding programmes. Identifying Quantitative Trait Loci (QTL) for strawberry quality traits will lead to a better understanding of how quality is regulated at the genetic level and how different traits are genetically correlated, facilitating molecular marker development. Therefore, to map QTL associated with the variation of shelf life and nutritional quality traits, measured over three post-harvest days in two sequential seasons, a linkage map based on 140 F1 individuals obtained from a cross between 'Redgauntlet' and 'Hapil' was constructed using 3933 SNPs distributed over 28 linkage groups. The map covers a total length of 2,624.7 cM with an average resolution of 0.7 cM/SNP. A subset of the population was grown in the field over two seasons at two different locations. The population showed transgressive segregation and a large range of variation between lines for each trait. We identified 47 QTL distributed over 20 linkage groups with an average explained variance of 18.8% and 19.9% for "year 1" and "year 2", respectively. Single Nucleotide Polymorphism (SNP) markers identified here and linked to the traits of interest are the first step towards improving strawberry marker-assisted selection programmes.